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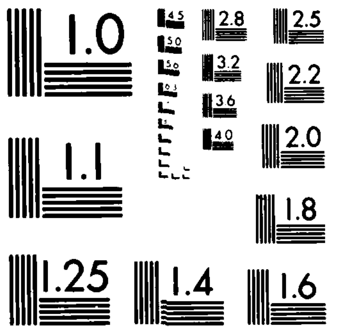

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The influence of chronic exposure to low level pulsed microwave radiation on performance and cognitive behavior

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MICROWAVE IRRADIATION AND OPERANT BEHAVIOR IN RATS:

FINAL PROGRESS REPORT

INTRODUCTION

Background -

Active projects in this laboratory have included the study of MWR interactions with the nervous system on the single cell level, on the biochemical level (that is, on neurotransmitter mechanisms and interactions with centrally acting drugs), and on the behavioral level. This last mentioned was the area of direct concern in this project support by ONR. After a brief description of the background and rationale for studying the effects of low-level MWR on complex operant behavior, I will outline the facility which had been developed to carry out such work. The main body of this report will consist of a summary of the results from our examination of the influence of rat operant behavior of acute and chronic exposure to moderate to low levels of microwave radiation (MWR).

The successful examination of the behavioral consequences of exposure to MWR we judged to be dependent upon the development of a specialized irradiation facility. This facility would have to satisfy several key criteria. Foremost would be the need to handle a large number of animals in virtually identical fashion so that statistically adequate control and experimental groups could be carried along in parallel. Secondly, it would be desirable that the behavioral testing and MWR exposure not be mutually exclusive, so that experimental designs could incorporate operant behavior coincident with as well as subsequent to irradiation. It also would be desirable that each rat be irradiated independently, that is free of interaction with its neighbors, that the irradiation levels be stable for all animals in a group and that there be minimal positional bias within the field. With respect to the behavioral apparatus, it would be necessary that within the confines of the waveguide exposure system the animals have minimal limitations on their normal range of motor activity (exploring, grooming and so forth). Considerable flexibility in control and data handling with respect to the behavioral components of the system also would be necessary in order to permit multiple experimental groups, each under a potentially different operant schedule, to be run each day.

An irradiation facility based upon individual waveguide exposure chambers (Guy and Chou, 1977) was judged best able to meet these criteria. To implement behavioral studies, operant manipulandum (bar press levers) and a food reinforcement receptacle were located at one end of each exposure chamber. The arrangement of these operant components was such that when actively engaged in bar-press, food retrieval and consumption the animal would be oriented toward the origin of the traveling wave.

Given the specialized input/output constraints, the large number of experimental animals and the need to tabulate operant behavior asynchronously and in parallel, a computer based data retrieval system clearly was required. The system was developed around an eight bit microprocessor augmented by readily available interface and support components. The input and output interface circuit modules were structured so as to be linked via an extended microprocessor bus structure to allow maximum expandability of the resultant system.

Perspective -

It is well known that innate patterns of behavior, such as locomotor activity, exploratory drive and emotional/autonomic responsivity, can be altered by exposure to relatively low levels of microwave radiation (MWR) (Eakin and Thompson, 1965; Korbel and Fine, 1967; Gillard et al., 1977; Hunt et al., 1975; Mitchell et al., 1977). The quantitative rigor of operant behavioral techniques proved particularly useful in developing a better understanding of the nature of the interactions of MWR with the nervous system and behavior (Justesen and King, 1970; Mitchell et al., 1977; Campbell and Thompson, 1975; Hunt et al., 1975). Although the absorption of MWR has evident thermal consequences, investigators had suggested that MWR can act as an operant cue at levels too slight to be associated with measurable changes in body temperature (Frey and Feld, 1975; King et al., 1971). Since suppression of operant responding can be observed subsequent to exposure at levels near current occupational guidelines (Gage, 1979), clarification of the critical underlying interactions is necessary.

The additional thermal load imposed by exposure to MWR certainly can be relevant to behavior. Sanza and deLorge (1977) had suggested that the levels of MWR exposure required for the suppression of operant responding were associated with measurable changes in body temperature. However, even with levels of exposure that imply thermal signals so small as to be comparable to the endogenous thermal "noise" of the nervous system (Howarth, Keynes and Ritchie, 1968) mechanisms had been proposed whereby these signals could become behaviorally significant (Adey and Bawin, 1977). To begin to resolve these issues requires, firstly, recognition of the fact that not all behavior is equivalently responsive to MWR (Thomas et al., 1975). Secondly, since some significant MWR interactions with the nervous system may be inherently transient, the conclusions from studies of changes in operant behavior subsequent to irradiation may differ substantially from those of similar studies carried out with concurrent irradiation.

The effects of microwave radiation (MWR) on physiologic processes continues to receive broad examination. Of particular interest in recent years has been the examination of the basis for MWR induced behavioral alterations (Adair and Adams, 1980; Blackman et al, 1980; Frey, 1977; Gage, 1979; Gage et al,1979). In this regard, key areas of concern include the accurate determination of threshold levels, determination of the nature of long term effects and of the effects of repeated exposure to MWR. The execution of credible investigations of the effects of low level MWR on animal behavior is complicated by several factors. First, there is the problem of the small magnitude and subtle characterization of behavioral alterations that appear to be elicited by subthermal levels of non-ionizing irradiation. Also, there is the nature of the MWR stimulus itself, viz., a propagating electromagnetic wave which precludes the use of traditional behavioral apparatus because of the induced electrical artifacts. In addition, the presence of conductors or large dielectric objects in the vicinity of the animal greatly complicates the control and verification of the microwave dose for that animal. Typically, the specification of MWR dose rate has been in terms either of incident parameters (field strength or incident power density) or of some measure of power actually absorbed by the test animal (power or energy per unit mass). The latter is currently the more generally accepted procedure (Joines and Blackman, 1980). With these factors in mind, we developed a multi-animal MWR exposure system with which to carry out long term studies of animal operant behavior coincident with prespecified levels of irradiation.

The behavioral alterations induced by exposure to MWR are, for the most part, readily reversible (Adair and Adams, 1980; Thomas et al, 1975). Since the absorption of MWR will always produce some degree of heating in the target body, immediate thermal factors provide a likely basis for such behavioral changes. However, changes in behavior have been reported using levels of MWR exposure that were not accompanied by measurable changes in body temperature (Frey, 1979;; Medici and Bawin, 1977). Adair and Adams (1980), for example, reported that even at 22 mW/cm^2, irradiated squirrel monkeys show no increment in rectal temperature if given the opportunity to regulate the ambient temperature. If they are intact, physiological and behavioral thermoregulatory mechanisms can operate with sufficient rapidity and accuracy to nullify the added thermal burden of whole body MWR (Gage, 1979; Stern et al, 1979).

Basic physiological variables have received extensive study and this may help to clarify the behavioral literature. Blackman et al. (1980) have confirmed and extended the work of Bawin et al (1975) regarding mobilization of brain calcium. The low levels of MWR required for enhanced calcium efflux is surpassed in potential significance only by the remarkable finding of narrow power density "windows" for the effect. Clearly, additional and varied confirmation of such data are in order. The studies by Tinney, Lords and Durney (1976) and by Reed, Lords and Durney (1977) have provided direct evidence for an influence of MWR on neurotransmitter release. Their work also supports the existence of amplitude windows, i.e. a narrow range of dose rate over which the responses can be observed. Sanders et al. (1980) provided a related observation that brief exposure to MWR at 5 mW/cm^2 decreases the levels of adenosine triphosphate and creatine phosphate in brain (with no detectable changes in brain temperature). Galloway and Waxler (1977) have reported that serotonin depletion in combination with MWR yields more disruption in behavior than does MWR alone. A serotonergic basis for behavioral suppression had been proposed (Cunitz et al., 1975); however, serotonin depletion itself is known to have profound behavioral consequences (Kiser, Lebovitz and German, 1978). Evidence that MWR may enhance the action of psychoactive drugs has been presented (Thomas and Maitland, 1979; Thomas, Burch and Yeandle, 1979), again, using MWR dose rates which cause no apparent change in whole body temperature.

However, the demonstration that intact animals can respond appropriately to MWR induced thermal cues does not mean that all behavioral effects must share this basis. Microwave hearing (Lebovitz and Seaman, 1977; Lin, 1977) and the influence of MWR on calcium dynamics of tissue (Bawin ad

Adey, 1976; Joines et al, 1980) are but two example of biological effects of MWR for which the modulation profile of the incident energy, not average dose rate (and hence, not steady state thermal factors), is the key factor. Given the large thermal inertia of the organ systems of even a small laboratory mammal, macroscopically detectable temperature changes would be expected to be directly proportional to the average rate of MWR absorption and thus independent of the form of the modulation of the incident MWR (Lebovitz, 1973). The utilization of MWR with diverse modulation profiles in conjunction with a set behavioral format is, therefore, an effective strategy with which to distinguish between the essentially steady state thermal and the transient or non-thermal modes of interaction.

LONG TERM GOALS - OVERVIEW

A focus of effort in this laboratory has been the study of complex operant behavior in rats during, as opposed to following, exposure to MWR. So that the neurophysiological, neurotransmitter and behavioral work underway here and in other laboratories can be put in some meaningful perspective, the aim of our effort was to develop a consistent set of conclusions regarding the dose/response relationships between MWR exposure and behavioral alterations. The design of the experiments was such as to obtain data at MWR dose rates for which thermal factors might be expected to be significant.

There is little doubt that thermal input can effect behavior. Whether by MWR, infrared, pyrogens or whatever, an added thermal load can impel the animal to adopt positive or negative strategies (depending on whether the animal's environment is such as to make the added thermal load a burden or a blessing). Likewise, thermal cues can modify innate behavior of a test animal or be used to shape its behavior. The demonstrated sensitivity of an animal to thermal factors will often depend on the sensitivity of the physiological or behavioral test set up to measure it. There was little advantage to our endeavors, therefore, in framing projects SOLELY in the context of "thermal" versus "nonthermal" factors associated with MWR. The basic questions, it seems to me, were (1) the magnitude of the lower limit on the level of MWR that can influence complex behavior and (2) whether the nature of any observed behavioral alterations were consistent with the MWR acting as an impediment to operant behavior or rather if we were dealing with a cue-like behavioral modification. The distinction does not arise when the levels of thermalization are quite high.

Emphasis has been placed upon simple behavioral protocols so as to be able to readily compare the results of our work with those already provided by other laboratories. The behavioral protocol involves fixed-ratio responding (FR) alternating with periods when instrumental responding yielded no reinforcement (time-out or TO). So as to be able to follow the longitudinal onset of any behavioral changes, each daily behavioral session was divided as follows: 15 minutes acclimitization, 15 minutes of FR-25, a 10 minute TO period, followed by five more FR,TO pairs and a final 15 minutes.

Early on we had found there to be a clear and regular effect of MWR using this simple instrumental format. There was a sharp difference between the influence of the MWR on the FR and TO behavioral components. At levels of irradiation that are significantly thermogenic (e.g., SAR of 6.8 mW/g) there regular but only small changes in the former compared with striking changes in the latter. A SAR could generally be found where the irradiated and sham irradiated animals were indistinguishable on the basis of the rewarded instrumental response rates, but their behavior during TO clearly differed. Since the fixed-ratio operant task yields highly stable behavior it was of interest to examine the influence of MWR on more flexible and responsive behavioral protocols. A modified differential reinforcement of low rate format (here actually a "counting" task, described in detail below) appeared well suited to providing the desired information and to be feasible with the behavioral system in place. It also provided a strategy for investigating whether the ability to learn and/or relearn specific operant tasks was modified by concurrent microwave exposure.

Our previous studies had shown that irradiated animals were less likely to bar-press during time-out periods (S^d, cue-off) than were their sham-irradiated counterparts. Since the time-out period took the form of a ten minute interval interposed between successive 15 minute fixed-ratio session components, the paradigm could also be viewed as a multiple schedule format of fixed-ratio followed by extinction. Under a repeated trials format we investigated whether there would be a corresponding tendency for emitted responses (bar-presses) to show an altered temporal distribution of responding compared with that of the control animals. Were such to be the case, then clearly it would imply that then examination of mean rates of responding is not be a sufficiently robust descriptive measure of the behavioral effects of MWR.

FACILITIES AND METHODS

MWR chamber -

The irradiation chambers were constructed of coarse (1/8 inch) metal mesh bonded to copper rings at either end. At the feed (or front) end of the chamber, the copper ring carried a pair of type N connectors to support the probes for launching the traveling wave into the structure. The rear section of the assembly was removable for insertion of a plastic animal housing and animal into the waveguide. This section also supported a pair of matched termination probes and was mated to the body of the waveguide via a fine mesh ring for secure electrical contact. s 8The overall length of the 15 cm diameter waveguide was 81 cm, with a usable interior length of 66 cm. The input and termination probes were located 7.5 cm from their respective ends of the waveguide cylinder. In most respects the design principles applied in the development of the waveguides were those presented by Guy and Chou, 1977. Operational frequency of our system was 1.3 Ghz, hence, the final available cross sectional area of the waveguide (177 cm^2) was somewhat smaller than that of the 918 MHz system designed by Guy and Chou (approximately 315 cm^2).

Energy was applied to the MWR irradiation chambers so as to launch a wave which has its E-vector in a plane orthogonal to the longitudinal axis of the cylinder and rotating in that plane (circularly polarized). To accomplish this, the nominal input MWR energy was divided by a 90^o hybrid (Microwave Techniques Inc., type B10207) and both components fed to the chamber via a pair of tuned probes. A total of 32 identical irradiation chambers are in use, with

16 used for irradiation and 16 for sham irradiation controls.

While in the waveguide, the animals were free to move within a vented plastic cylinder, 14.6 cm in diameter and 30.5 cm long. The design of this inner housing allowed for ready access to presented stimuli, manipulanda and rewards but prevented the animal from contacting any portion of the irradiation system. The inner cylinder floor was constructed of coarse plastic lattice that allowed the animals' excretions to fall to a water-tight area 4 cm below the support plane of the animal.

MWR source -

Pulse modulated energy at 1.3 Ghz was supplied by a surplus Navy RADAR source (AN/SPS-6C) operating in narrow pulse mode (1 microsecond pulse width) at 600 pulses per second and capable of producing a peak pulse power of 750 KW. The MWR energy was coupled via L-band waveguide to a waveguide attenuator (MTI type WR650) capable of inserting up to 20 dB additional power loss in the main feed. The attenuated pulse modulated energy then drove a divide-by-16 power splitter (MTI "Cobra") that provided the sixteen pairs of dual chamber feed lines. Each main feed line was provided with forward and reflected power sampling ports (MTI bidirectional couplers type B10259). The output of this power divider was coupled to the irradiation chambers by equal lengths of RG-214/U 50 ohm cable (Belden type 8268) so as to provide equivalent phase shift and attenuation in each feed. In addition, the transmission characteristics of each cable of the array were tested and matched to within 10%. Each cable also was checked for proper termination so that none presented a VSWR of more than 1.1 at either end.

Continuous wave MWR was supplied by an MCL RF power generator (Model 15022) capable of 100 watts CW, with auxiliary cooling. PM and CW irradiation were carried out separately, with each source appropriately connected to the MWR divider network for power distribution to each of the irradiation chambers (active waveguides).

In the case of the PM-MWR, source power was variable only in discrete steps (1:1.09) and then only for the entire array as a unit. In addition, post-irradiation calibration (especially at higher intensities) occasionally yielded actual dose rates somewhat different from the target value. It was a matter of protocol not to adjust SAR once a given experiment component had started. For these reasons nearly equal dose rates for different groups of rats had to be accepted where numerically equal value would have been the ideal.

During behavioral sessions, power levels were monitored at the divider input (net input power) and at the output to two or more chambers (sampled output power) at the divider outputs. The total attentuation in the feed cables being known, this provided a sample of the chamber input power. For calibration of SAR, however, power measurements were done using power meters and dividers located at the input and termination ports of the irradiation chambers (see below, Dosimetry). The entire array was checked on a regular basis with a broadband, isotropic radiation monitor (Narda 8305). The minimum detectable power density with thus instrument was .02 mW/cm^2 and system operation would have been suspended if leakage in excess of .05 mW/cm^2 were found. This never occurred with the array used for behavioral trials.

Behavioral array -

The 32 irradiation assemblies were arranged in three free-standing wooden racks, with control (sham) and energized chambers randomly placed within the array. The plastic animal housing slipped into the bore of the waveguide chamber and mated with the permanently mounted non-metallic bar press and food reward tray. The feeder apparatus (Gerbrands pellet dispenser) was located adjacent to each waveguide and delivered a 45 mg Noyes food pellet via a plastic tube passing through a small hole at the side of the waveguide. Visual discriminative stimuli were presented by projection onto the opaque plastic wall that formed one end of the animal housing. The movement of each manipulandum was coupled photo-optically to a coded input port of the microprocessor controller. No provision for water intake was made, hence, the behavioral sessions were kept sufficiently short to maintain steady bar press activity for food reward throughout (Lebovitz, 1980).

In terms of the overall structure of the computer system, each of the 32 chambers was serviced in a asynchronous and independent fashion. That is, each stimulus presentation, bar press lever and pellet dispenser was accessible via separately dedicated ports. Each bar press was logged in terms of chamber number and absolute time, with a maximum, non-accumulating error of 40 msecs. Reward schedule and session sequencing were determined either on-line by the operator or prescheduled via software stored on floppy disk.

Since the difficulty of providing correct MWR dosimetry has contributed only confusion to the current literature with respect to behavioral effects, the development of a method for non-restrictive, non-invasive measurement of whole body average dose rate per unit mass (specific absorbed-dose rate, or SAR) by Guy and Chou 10 is highly significant. In essence, the animal is placed in a waveguide instrumented so as to be able to measure input power, reflected power and power transmitted past the animal. From a simple power balance relation (correcting for inherent losses of the empty waveguide), the net power absorbed by the animal at any given moment can be determined. Although animal movement will affect the moment to moment SAR, energizing the exposure waveguide with a circularly polarized traveling wave significantly reduces the effect of animal posture on its absorbed dose rate 10 .

We have assembled a system of 32 such waveguide sections into a randomized array of 16 active plus 16 sham irradiation units. In order to achieve maximum flexibility with respect to the MWR parameters, the facility is designed so that all of the active chambers can be driven in parallel from a single power source. In addition, each waveguide irradiation cell is provided with an inner behavioral subassembly of thin plastic and glass which incorporates a simple operant task (bar press), operant reward delivery (food pellet) and discriminative stimulus presentation (visual). Control and data flow in the array is managed by an inexpensive microprocessor system augmented with behavioral input/output interfaces specifically designed for this application.
elsewhere (Lebovitz and Seaman, 1980). Animals were irradiated, each in their individual waveguides, using pulse modulated (PM) MWR at 1.3 Ghz with the pulse width fixed at one microsecond and at a pulse repetition rate of 600 per second. The MWR dose rate was specified in terms of the whole-body, specific absorbed dose rate (SAR), in mW/g. The irradiated and control groups were

always run simultaneously, with all rats in their respective, identical exposure chambers. However, to control for any positional bias in the array, the rats were randomly reassigned to new chambers each week. Temperature and humidity were noted daily. The latter was unregulated and varied over the range of from 40 to 60%, with infrequent extremes of 20 and 80%. Room temperature, however, was regulated to $21 \pm 1.5^{\circ}C$.

Animals and behavioral procedures -

Groups of Long-Evans hooded rats were obtained from an established vendor colony (Blue Spruce Farms) at approximately 40-60 days of age. After acclimatization in individual housing for two weeks with food and water ad lib, the animals were food deprived to 85% of their ad lib body weight and shaped to bar-press for food pellet reinforcement. This shaping progressed from a fixed-ratio of one (FR-1) through FR-5, whereupon the animals were introduced to a multiple component schedule. Under this schedule, each daily operant session started with a fifteen minute period during which the visual discriminative stimulus lamp was on (S^+) and food reinforcement was available at FR-25. This was followed by a ten minute timeout period during which the visual discriminative stimulus lamp was extinguished (S^-) and responding was recorded but not rewarded. The sequential S^+, S^- component periods were repeated five additional times - 6(FR(25) 15, TO 10). A fifteen minute null interval (discriminative stimulus extinguished, responding not tabulated) preceded the start of the first S^+ component period and followed after the last S^- component period, for a total behavioral session of three hours in duration. A single priming pellet was delivered to each animal coincident with the first onset of the discriminative stimulus.

All animals were run once per day, at the same time each day, 5 days per week. When not under test the animals were kept in individual home cages with water available ad lib. Each animal received a food supplement consisting of 8 grams of standard laboratory chow once per day, independent of operant performance. The animals were weighed three times per week. No animal failed to maintain a satisfactory growth curve under this protocol; one animal was lost due to a malfunctioning automatic water supply and its data were deleted retroactively.

Each experiment consisted of subjecting previously trained rats to MWR at a predetermined and fixed dose rate, in the following fashion. Once the group of 30 animals had reached a high and steady level of bar pressing at FR-25 (an average of more than 4.5 grams of food earned operantly per day) a baseline period began. During baseline the MWR source was on but set to zero output power. At the end of baseline the animals were randomly assigned to irradiated or control (sham-irradiated) groups of 15 animals each, with the two groups matched on the basis of baseline FR-25 performance. The irradiation phase of each experiment then proceeded for 6 to 9 weeks. Each experiment concluded with a recovery period of at least two weeks, during which the rats were run as during baseline, viz., with the MWR source on but with no energy delivered to any waveguide chamber.

After shaping, groups consisting of up to 30 rats were run once per day until an average of 500 food pellets was obtained operantly per rat per day. This took several weeks, by which time the animals were responding at a high and steady rate during the S^+ component periods. Following a three-week baseline phase, the rats were assigned to irradiation or sham irradiated (control) subgroups so as to have comparable group average S^+ baseline response rates. The animals remained on the 6(FR(25) 15, TO 10) schedule throughout the irradiation phase.

When the protocol called for MWR, it was applied during the entire three hour behavioral session. To provide for a uniform level of background noise, the MWR source was on at all behaviorally significant times, whether MWR energy was being supplied to any chamber or not. Temperature (23 +/- 1.5°C) and humidity (50% +/- 15%) were noted daily, before and after each run.

The animals were kept on a 12-12 light-dark cycle (lights off at 7 am) and run at the same time each day in their dark phase (between 9 am and 2 pm). Each animal was provided with a food supplement of 6 grams per day, independent of its operant performance.

Data analysis -

A detailed description of the computer based control and data handling system has appeared (12). The key points to note here are that (a) the operant sessions ran concurrently with the microwave exposure and (b) the irradiated and sham-irradiated animals were run simultaneously, i.e., in parallel. The primary data for each rat was the number of bar-presses emitted per S^+, S^- component period. Weekly performance was summarized by totaling the responses emitted by each rat over the five corresponding daily sessions for each behavioral component period and converting this total to a weekly individual response rate (bar-presses per minute). Three-factor analysis of variance (ANOVA) with repeated measures provided the basic estimates of statistical significance. The repeated measures were behavioral component period (FR or TO 1 through 6) and week (pre-MWR versus MWR); the third factor was treatment (sham versus actual MWR). Two-factor ANOVAs (with repeated measure on the intra-session component period) were applied to probe for the sources of significant variation due to factors or to factor interactions. In view of potential rate effects, non-parametric group (Mann-Whitney U) and by subject (Wilcoxon signed-ranks) statistical tests were used for subsequent pair-wise examination of particular differences between the mean response rates.

RESULTS

Electric field characteristics of the exposure chambers -

The operational frequency of 1.3 Ghz was selected on the basis of several factors: relevance of this frequency to actual human exposure profiles (RADAR), wavelength relative to animal size (for increased absorption efficiency) and ready availability of high power, pulse modulated (PM) power sources. The latter was a key factor since a comparison between the actions of PM and continuous wave (CW) MWR was desired. The need to maintain uniform, single mode transmission within the waveguide had to be balanced against the need for a waveguide diameter sufficient to accommodate a 200-500 gram rat.

In a cylindrical waveguide, the dominant mode of wave propagation is the TE_{11} mode, for which the cut-off frequency is

$$f_c = 1.841 \left(c \,/\, (\mathrm{pi})\ d\right)$$

where c is the velocity of light and d is the interior diameter of the waveguide (Adam, 1969). For a cylindrical waveguide 15 cm in diameter, f_c = 1.18 Ghz. The cutoff frequency for the next lowest mode of propagation (TE_{21}) is 1.95 Ghz. Hence, single mode propagation within the waveguide was assured.

The unitized waveguide exposure system was designed so as to expose each individual test animal to a unidirectional, circularly polarized traveling wave. One measure of the satisfactory operation of the exposure system was the interior field distribution under various conditions of interior loading. This was examined using a non-perturbing microwave power density probe (kindly furnished by H. Bassen of BRH, cf. Bassen et al, 1975). A three axis carrier was built to hold the test irradiation chamber and to allow rapid and repeatable placement of the electric field probe tip at specified positions within the waveguide.

The results of these measurements are summarized in Lebovitz and Seaman (1980, see appendix). In brief, the variations in field intensity along the axial length of the empty waveguide were small and significantly perturbed by the animal but not by the animal carrier or behavioral apparatus.

VSWR was measured along a vertical plane through the longitudinal axis of the chamber and 1.5 cm above center in order to approximate the longitudinal axis of an adult rat placed within the guide. The VSWR of the empty waveguide was calculated to be 1.20 (1.57 dB). With the behavioral apparatus and animal chamber in place but empty, there was some shift in the residual standing wave pattern but little change in the VSWR over the working area of the waveguide (VSWR = 1.23 or 1.8 dB). The VSWR was not significantly changed when the nominal input ports were terminated with 50 ohm passive loads and the nominal termination ports were used as inputs. That the transmission characteristics of the chamber load waveguide were symmetrical was an additional substantiation of satisfactory design of the waveguide chamber and the input/output probes. The presence of an adult rat carcass of approximately 300 grams in the waveguide resulted in a substantial elevation of the VSWR to 1.79 or 5.05 dB.

On the basis of these data and upon the data reported in Lebovitz and Seaman (1980) we concluded that the interior field was uniform, minimally affected by the animal carrier and behavioral apparatus, and well coupled to the test animal.

Several experiments were undertaken to insure that the microwave dose rate and field profiles were as expected. These included basically electrical measurements such as field strength and VSWR. The effects of the animal housing and the behavioral apparatus (both constructed of thin plastic) were found to be minimal. There were no significant variations in net absorption profile in the rat with longitudinal placement in the chamber (posture and orientation held constant). Four-port measurements on the waveguide irradiation chamber indicated that the rat absorbed 35-50% of the incident MW energy. This was somewhat higher than the percentage reported by Guy and Chou. However, it can be noted that, with respect to the Guy et al., system, the higher operating frequency of our system required a slightly smaller waveguide. Hence, a larger fraction of the cross sectional area was occupied by the test animal.

Animals tolerated the behavioral apparatus quite well. There was every indication that the size, ventilation, isolation, and so forth, of the individual chambers within the behavioral array were appropriate for behavioral studies. Of more direct interest, however, was the relationship between chamber input feed power and absorbed dose rate for the test animals.

Dosimetry -

An advantage of the waveguide exposure system is that absorbed dose rates can be obtained from multiport power measurements at the chamber with the animal unrestrained. While this procedure avoids the errors inherent in incident field measurement schemes, it presents the disadvantage of yielding only the whole body average SAR. To obtain a more detailed appreciation of the exposure conditions measurements of whole body SAR, regional SAR and estimates of the effective incident field intensity were made utilizing rats and small volume saline loads. The expression for the net power absorbed by a test animal is:

$$P_a = P_{in} - P_r - P'_r - P_t - P'_t - P_l$$

where the subscripts r and t indicate reflected and transmitted power, respectively, the 90° phase shifted components are primed and P_l is the intrinsic loss term for the empty chamber. Calibration was accomplished using continuous wave (CW) MWR at 1.3 Ghz (MCL type 15022 power source, Narda integrated thermocouple type 462, TRM 90° hybrids type 50021, AL circulator type 100100007 and Narda coaxial direction couplers type 3002-30) using the methods outlined in Lebovitz and Seaman (1980).

Time-temperature increment data were derived for several positions along the longitudinal axis of the exposure system. The normalized SAR_T for these saline targets near the front, middle and rear of the chamber were calculated to be 2.0, 2.3 and 2.0 mW/g per watt input respectively. The corresponding SAR_D's, derived directly from simultaneous port measurements, were 2.0, 2.6 and 2.1 mW/g per watt input. While there was good agreement, the multiport measurements appeared to consistently overestimate the thermometrically derived SAR by a small margin. The largest discrepancy was behind the front of the behavioral chamber, at the broad local field maximum as determined by the Overall, the average difference between SAR_T and SAR_P was less than 6.5%. As a conservative estimate of the exposure conditions for the purpose of subsequent behavior studies, the higher figure was used for the determination of exposure conditions. The limit of accuracy of our MWR calibration procedures should be placed at 10%.

To examine the relevance of the SAR_P as an estimator of dose rate in test animals, a rat carcass was placed at the front of the behavioral chamber, elevated and oriented by small Styrofoam blocks to be in the approximate position of an animal active at the behavioral manipulandum. Under these conditions the normalized whole body SAR_P was 2.2 mW/g per watt input, which agreed closely with the SAR_T described above. Time-temperature measurements of regional SAR using methods analogous to those described above for the saline load experiments yielded a head SAR_T of 7.9 mW/g per watt input as compared to a hindquarters SAR_T of 1.3 mW/g per watt input. The ratio of peak to average SAR, therefore, was at least 3.5 to 1.

In summary, whole body SAR_P was in close agreement with SAR_T obtained with saline loads. Furthermore, saline load SAR_P and SAR_T were in close agreement at various discrete points within the waveguide. Regional SAR_T in the rat, although not intended as a specified independent variable for these studies, was consistent with the expected regional variations in the rat at 1.3 Ghz (Durney et al, 1978; Guy and Chou, 1977). These data thereby established the validity of the SAR_P as a consistent estimator of dose rate in the waveguide array.

Animal activity -

If a large number of chambers are to be in use, there would be considerable advantages in cost and efficiency if the SAR_P could be shown to be invariant or at least stable despite animal movement. To investigate this, the SAR_P was displayed on a polygraph while test animals were allowed to roam freely and to actively bar-press for food reward (cf., Lebovitz and Seaman, 1980). Estimation of the time average of SAR_P over ten minute intervals (i.e., intervals long with respect to animal movement episodes) indicated that the SAR_P seldom exceeded 2.2 mW/g per watt input and was virtually always within 10% of 2 mW/g per watt input. Again, therefore, it appeared that the former value was a proper, conservative estimate of the imposed dose rate characteristic of the irradiation array. In trial runs using active rats. no significant effect of animal movement in one chamber on the SAR in another chamber could be measured. Direct measurements of the amplitude balance showed that the mean amplitude balance among the feed ports was +,- 6.14% or +,- 0.27 dB. Specified SAR therefore represents an average SAR over all of the simultaneously exposed rats receiving a given dose rate.

Operant behavioral changes associated with exposure to PM-MWR -

When deprived of food so as to yield a weight loss of approximately 15% and then given the opportunity to establish fixed-ratio (FR) operant responding for food reinforcement, most animals acquired this skill within two 3-hour sessions. Additional shaping and final selection of the experimental group took 3-4 weeks. Training under the multiple (FR-25, extinction) schedule then proceeded for at a variable number of weeks, generally four, before beginning the behavioral baseline. The aim was to insure responding that during S^D (FR) attained relatively high and stable levels. S^Δ (TO) response rates fell to a stable level of approximately 10% of the S^D rates before the baseline period was initiated. The multiple FR-25, TO-10 schedule was utilized for a large portion of the behavioral work. Its advantages included a stable level of performance for bulk operant measures, compatibility with detection of intra-session as well as between session (longitudinal) and treatment trends and, finally, the strong contrast between a robust schedule controlled behavior as compared with a relatively non-controlled, low response rate behavior.

Figure 1 summarizes the effect of MWR at an SAR of 1.5 mW/g on FR-25 (S^D, upper) and time-out (S^Δ, lower) operant response rates. Session blocks (or component periods) 1, 3 and 6, correspond to the beginning, middle and final segments of the behavioral session, over the 13 weeks duration of this experiment; total session responding (the sum of session component periods 1 through 6) are shown in upper and lower panels A. Since the control and irradiated animals were selected on the basis of group matched levels of performance during baseline, a three-factor analysis of variance with repeated measures on week and block (RM-ANOVA) was applied to the data of the eight irradiation weeks.

Several basic findings are illustrated by the graphed and statistical data. Rewarded operant behavior was stable throughout. There was a slight but significant upward trend in the mean FR response rate over weeks ($F(7,196)=2.79$, $p=.009$). This was most evident early in the sessions and would suggest that the animals were becoming more proficient at the task and/or motivated to consume more food. The animals showed their highest rate of operant response also during the early part of the session (compare Fig. 1, upper panels B, C and D). However, the animals were still highly active at the food-reinforced, FR-25 task at the end of the 2-1/2 hour operant session.

The stable rate of FR operant responding and its modest decline over the course of each daily session were consistent findings throughout all of the studies in which FR schedule control was used. At 1.5 mW/g there were no significant group-dependent interactions nor any main effect by group to suggest an effect of the MWR on FR response rates. The significant interaction between week and block ($F(35,980)=7.63$; $p<.0005$) indicated that the rate of decline of FR responding during each session was not uniform over the irradiation weeks. Although no group dependent interactions were evident, an effect of irradiation on this daily, intra-session decline in performance would be an interesting finding. Therefore, a two-factor RM-ANOVA by group (i.e., by treatment with repeated measure on blocks) was applied to the data of each irradiation week. For no week was there a significant difference between the groups. This was consistent with the more inclusive three-factor ANOVA. The conclusion is that whereas there were significant variations from week to week in the details of FR operant responding, these differences did not derive from the irradiation. Control and irradiated groups maintained acceptable and indistinguishable average growth curves throughout the experiment.

Response rates during TO were considerably lower and more variable than during FR-25 operant component periods. Again, there was a slight trend over the course of the experiment indicative of longitudinal as well as intra-session variations in behavior. However, in this instance the variation over weeks was not significant ($F(7,196)=.536$; $p=.580$). The decline in TO responding over the course of each daily session ($F(5,140)=26.72$; $p \le .0005$) was much sharper than that for FR responding. TO response rates near the end of the sessions were typically 10-20% of of those at the beginning (compare Fig.1 lower panels B, C and D). The lack of significant group-dependent main or interaction effects suggested that MWR at 1.5 mW/g did not influence S^d responding.

The experiment was repeated using another group of rats and again no detectable effect on FR or TO response rates resulted over an eight week exposure period. We therefore took 1.5 mW/g to be below threshold under this behavioral protocol.

The intra-session variation (block effect), seen with respect to FR and TO performance, consisted of progressive declines in the respective response rates over the course of each daily session. This was evident in all experiments and very likely was inherent to the behavioral format. The term "extinction" may be applied to the decline in TO responding; "satiation" may be more appropriate in referring to the corresponding decline in FR responding. Recognize, however, that the FR and TO session components formed an alternating sequence. This very regular pattern of intra-session performance should not be confused with longitudinal changes in response rates that appeared over the duration of a given experiment (2-3 months).

The effects on FR and TO response rates of exposure to PM MWR at 3.6 mW/g are shown in Figure 2, in the same format as used in Figure 1. After a three week baseline period the irradiated group experienced MWR for nine consecutive weeks, which period then was followed by a two week recovery period (no MWR). As in the experiment at 1.5 mW/g, FR operant behavior decreased over the course of each session ($F(5,130)=77.28$; $p \le .0005$), but was otherwise stable for the duration of the experiment. There were no evident group main or group-dependent interaction effects of the MWR on FR responding, although there was a marginally significant variation week to week ($F(7,182)=1.84$; $p=.082$). Two-factor RM-ANOVA's by group (as above) for each irradiation week did not uncover any potential treatment dependent differences in mean FR response rates or rates of satiation. The weight curves indicated stable, steady growth with no differences between the two groups that could be attributed to their irradiation history.

The within-group variability of the rate of TO response rates again was considerably greater than that for FR responding, for the non-irradiated as well as irradiated animals. Also, it again was evident that the TO response rates declined sharply over the course of each daily session ($F(5,130)=29.75$; $p \le .0005$). In addition to a significant variation over weeks ($p=.003$) there was a marginally significant three-way interaction between group (treatment), week and block ($F(7,182)=3.26$; $p=.088$). Whereas the former could have reflected changes in behavior in both the control and irradiated groups over the course of the experiment, the latter suggested that the irradiation may have had a specific effect over weeks and/or within session. The plotted data did, in fact, appear to indicate that during the first week of MWR the irradiated rats showed a reduced rate of TO responding. To examine this more closely, a detailed statistical analysis was performed, again using two-factor RM-ANOVA applied to the data of each of the irradiation weeks. The differences in responding between week 3, the last week of baseline, and week 4, the first week of MWR, were analyzed via two-factor RM-ANOVA. This incorporated comparisons by subject as well as by treatment, thereby making use of the fact that each rat also served as its own control.

In summary, the results showed that there was no statistically significant overall difference between the groups ($F(1, 26)=2.67$, $p=.113$) but a highly significant two-way interaction (group by intra-session time course: $F(5, 130)=3.94$, $p=.003$). Simple statistical comparisons of the mean bar pressing rates during the first week of irradiation indicated that the irradiated group responded at significantly lower rates than the controls by midway through each session. Thus, the rate of extinction of TO responding appeared to be enhanced during the first week of irradiation.

In a repetition of this experiment with another group of Long-Evans rats, essentially the same results were obtained. The only reliable behavior changes observed was in the TO component. It is reasonable to conclude that, whereas there was no significant effect of the MWR on overall FR-25 or TO responding, there was an enhanced rate of extinction of the latter response component during the early phases of irradiation. As discussed below, similar experiments using CW MWR supported these data and confirmed that TO response rates were a more sensitive indicator that FR-25 responding of potential disruption by MWR.

At 6.7 mW/g, there were quite evident effects of the MWR on FR and TO responding. Figure 3 shows the course of the eleven week experiment. Satiation was evident in the behavior of both control and irradiated animals. In addition, however, the irradiated animals appeared to show reduced overall FR responding relative to the controls. The magnitude of this difference was relatively small, but indicative of a persistent reduction in cued, food-reinforced bar pressing behavior. The raw data appeared to suggest also that this effect developed gradually over the first two to three weeks of irradiation. The irradiation phase was cut short at these high dose rates in view of the obvious behavioral alterations and thermogenic nature of the dose.

There was no statistically significant difference in overall FR responding between the irradiated and the control group. There were, however, significant intra-session interactions. The source of these interactions was sought via two-factor RM-ANOVA by group applied to the data of each of the irradiation weeks. In summary, while no significant differences in overall responding by group were found, a significant two-way interaction (between group and intra-session time course) was found in the second irradiation week ($F(5, 135)=3.05$, $p=.012$) and marginal two-way interactions ($p=.07$) in the first, third and fourth week of irradiation.

When taken together, the above results tend to indicate that the irradiation caused an enhancement of the rate of satiation, but that this did not appear immediately, even at 6.7 mW/g. Rather, this developed over several weeks of daily exposure.

The changes in TO responding at the onset of MWR were quite apparent (Fig. 3, lower). A three-factor RM-ANOVA applied to the data of the 6 week irradiation period indicated a significant difference between control and irradiated groups, as well as significant two way interactions (group by week and group by block). The latter, in particular, supported the conclusion that there was a significant reduction in overall TO bar pressing at 6.7 mW/g as well as an enhanced rate of extinction. The reason for the abrupt increase in S^d responding by the control animals upon the onset of MWR was not apparent. In no other experiment was such a marked change seen in the control group coincident with a change in MWR status. However, a two-factor RM-ANOVA by subject indicated that for the irradiated group the overall S^d responding during the first week of irradiation was reduced and the rate of extinction increased with respect to the performance during baseline (overall: $F(1, 14)=4.74$, $p=.045$; extinction: $F(5, 70)=4.15$, $p=.003$).

A brief confirmatory experiment with another group of animals irradiated at 6.7 mW/g yielded essentially the same findings with respect to the MWR induced slight reduction in and major reduction in TO response rates.

At the start of the post-MWR recovery period, there appeared to be a substantial rebound in the level of S^d bar pressing by the animals of the irradiated group (week 9). A three-factor RM-ANOVA applied to the data of the offset transition weeks (last week of MWR versus the first week post-MWR) indicated a significant three-way interaction (group by week by block: $F(5, 135)=4.50$, $p=.001$) despite the evident dilution of the irradiation factor (that is, neither the control nor the "irradiated" animals actually received irradiation during the recovery weeks). Therefore, a less general, two-factor RM-ANOVA by treatment was also applied, as above, on the differences (by rat) in bar pressing between weeks 8 and 9. Compared with the control group, the irradiated group showed a marginally significant rebound in TO response rates upon the offset of MWR ($F(1, 27)=3.79$, $p=.059$) and a highly significant reduction in the rate of extinction ($F(5, 135)=4.50$, $p=.001$). Thus, during the first week post-MWR, the rate of S^d response extinction appeared to decline, as if TO responding was released by the cessation of MWR. Two and three-factor RM-ANOVA's by treatment indicated that there were no significant differences in S^d responding between control and irradiated rats during the period of post-MWR recovery. This is a minor point, and would be more significant if the rebound was to levels in excess of the pre-MWR baseline. As this was not the case, the observation simply illustrates the ready reversibility of even dramatic changes in operant behavior following exposure.

Operant behavioral changes associated with exposure to CW-MWR -

At an average-SAR of 5.9 mW/g, FR and TO responding were substantially reduced by CW MWR. In most respects, the behavioral changes coincident with exposure to CW MWR were comparable to those elicited by exposure to PM MWR, as presented above. It is convenient for this comparison, however, to focus attention on the first week of irradiation. Figure 4 illustrates this by comparing the behavior of groups of 15 control and 15 irradiated animals during the first week of MWR with that of the preceding week. The effect of MWR on the two behavioral components will be considered separately.

During baseline, the response rates under FR schedule control showed a moderate decline of approximately 10% over the course of the behavioral session (Fig. 4A, Pre-MWR). Three-way ANOVA (pre-MWR versus MWR by component period by treatment) showed that the decline in FR responding over the course of the daily sessions (FR component periods 1 through 6) was a statistically significant major factor ($F(5, 140)=50.97$, $p \leq .0005$). This effect of satiation was apparent under all treatment conditions and for all subgroups in the experiments discussed in this report.

Upon exposure to CW MWR (Fig. 4A, MWR), the initial response rates were unchanged. However, the rate of decline was substantially increased. By the end of the session the mean rate of responding by the irradiated animals had fallen considerably below that for the previous (baseline) week as well as below that for the parallel controls. The three-way ANOVA revealed a significant interaction among all three factors ($F(5, 140)=3.57$, $p=.005$) despite the considerable dilution due to the fact that no MWR was applied to any animal during baseline.

The results of two-factor ANOVAs (by treatment, with repeated measure on component period) indicated that during baseline there were no significant differences between the subgroups nor any significant interactions between treatment and component period. During the subsequent week of MWR exposure, while there was only a marginally significant difference in overall responding by treatment ($F(1, 28)=3.38$, $p=.073$), the treatment by component period interaction was quite evident ($F(5, 140)=7.53$, $p \leq .0005$). The results of pair-wise, non-parametric tests of the significance of the differences in performance by component period verified that there was a significant reduction in the rate of FR responding by the irradiated subgroup towards the end of the operant session. From these statistical results and from the form of the FR response profiles it follows that, while no change in total responding could be shown to derive from the MWR, the 5.9 mW/g dose rate was associated with a clear increase in the rate at which FR responding declined over the course of the session (increased rate of satiation).

It was apparent that the pattern of TO responding (Fig. 4B) was altered markedly coincident with the onset of MWR at 5.9 mW/g; it was essentially reduced to zero by the end of the behavioral session. A three-factor ANOVA revealed a significant interaction among all three factors ($F(5, 140)=2.91$, $p=.015$) along with significant week by component period ($F(5, 140)=3.45$, $p=.006$) and week by treatment ($F(1, 28)=10.47$, $p=.003$) interactions. The progressive decline in TO responding over the course of the session (extinction) was a major factor ($F(5, 140)=9.41$, $p \leq .0005$) independent of treatment and, as with the intra-session decline in FR responding, apparent in all the subgroups of this study.

Two-factor ANOVAs verified that during the pre-MWR week there were no treatment dependent main or interaction effects. The MWR exposure, on the other hand, coincided with both a decline in total TO responding (treatment effect: $F(1, 28)=16.72$, $p=.0006$) and an enhanced rate of extinction of TO responding (treatment by component period interaction: $F(5, 140)=6.11$, $p \leq .0005$). The results of non-parametric tests of differences in responding by component period verified that this enhanced rate of extinction was significant by the second TO component period, that is, 40-50 minutes after onset of irradiation in a session.

With CW MWR at an SAR of 3.6 mW/g (Fig. 5) a three-factor ANOVA revealed no significant MWR related effects or interactions with the FR component periods.

A three-factor ANOVA of TO responding yielded no significant three way interactions, but indicated significant week by subgroup ($F(1, 28)=8.27$, $p=.008$) and week by component period ($F(5, 140)=2.62$, $p=.027$) interactions. The graphed data (Fig. 5B, MWR) suggested that there was an enhanced extinction of

TO responding. Two-factor ANOVA by subject (i.e., pre-MWR versus MWR) suggested that for the irradiated subgroup the decline in overall TO responding was significant ($F(1, 5)=14.2$, $p=.002$). The source of the interactions appearing in the three-factor ANOVA thus appeared to be the relatively low TO response rates of the irradiated subgroup late in the sessions. The results of non-parametric pair-wise comparisons supported the suggestion that the TO responding of the irradiated subgroup late in the session was reduced with respect to the performance of the controls as well as with respect to their baseline performance. Clearly, while marginal, these changes are sufficient to indicate that the 3.6 dose rate was approximately threshold under this multicomponent schedule.

Comparison of the results of exposure to PM versus CW MWR -

A total of ten groups of animals were run in a total of twelve experiments, each incorporating at least one and generally three months of study of fixed-ratio operant behavior coincident with MWR. While limitations in source power flexibility did not permit exact duplication of SAR's a good comparison of the effects of CW and PM MWR could be made. Figures 6 and 7 illustrate the relative changes in FR-25 response rates during the first week of irradiation (R_i) relative to the previous week of baseline (R_b) as a function of FR component period, with SAR as a parameter. The function plotted is $(R_i/R_b)-1$. The experiments using PM MWR were run early in the project and our procedures had improved considerably by the time we approached focused comparative study of CW MWR. Hence, the PM data of Fig. 6 were somewhat "noisy". The general conclusion were clear, however. In order to have a significant influence on FR-25 response rates at an SAR on the order of 6 mW/g, an exposure of an hour or more was required. At 3.5-3.6 mW/g, a decline in FR-25 rates was observed after 2 or more hours of exposure. The PM and CW data were essentially in agreement.

While the animals exposed to 1.5 mW/g PM MWR appeared to have yielded a reduced FR-25 response rate early in the session, this difference was not significant. In our judgement this is an anomolous result in that it not fit the pattern yielded by all other groups exposed at this dose rate. We include the data here for uniformity of overall exposure durations; it is a good illustration of the absolute requirement for multiple experiments using large numbers of parallel and self-controlled animals.

Despite the higher variability of TO response rates, the similar changes in this output variable subsequent to PM and CW irradiation was quite convincing (Figures 8 and 9). For both form of irradiation, 3.6 mW/g was an apparent threshold dose. Suprathreshold dose rates yielded a prompt and sharp reduction in TO response rates, but still not during the first TO component period. That is, an exposure duration of at least 40 minutes (under the protocol of these experiments) was required before a significant reduction in TO response rates was observed.

In summary, at SAR's suprathreshold for an effect on both FR and TO response components in this multicomponent schedule, the results of PM and CW MWR were qualitatively indistinguishable. Any explanatory mechanism accounting for these behavioral results at suprathreshold MWR dose rates would have to take into account a primary dependence upon average and not peak absorbed-dose rates.

Despite some obvious dissimilarities in baseline performance between the experimental groups, CW and PM MWR at 3.6 mW/g appeared to have essentially the same differential influence on FR and TO responding. It is doubtful that a single bulk measure of behavior or short operant sessions (under 40 minutes in duration) would have been adequate to demonstrate these effects.

Onset of MWR-induced changes in operant responding -

The above data have been presented in terms of a comparison of the weekly average response rates. When viewed on a finer time scale, the data revealed a distinct difference between the mode of onset of the alterations in FR and TO responding. Figure 10 illustrates the FR and TO response profiles for selected portions of each daily run (component periods 1,3 and 6, corresponding to the beginning, middle and end of session) before and during the exposure to MWR. The bottom panel in each column summarizes the overall response rates for that component of the schedule (FR or TO). FR responding (Fig. 10A) was relatively regular over the entire baseline week, with even the somewhat reduced rates of response by the end of the session (FR component period 6) regular across subgroups and days. The irradiation (here CW at 5.9 mW/g; 15 control and 15 irradiated animals) yielded a gradual decline in FR response rates over three days. The apparent subsequent recovery was accentuated by an unexplained drop in FR response rates of the control group on day 10. In fact, the FR response rates of the irradiated rats remained below baseline throughout subsequent second and third weeks of irradiation at this same dose rate (not shown). Note that the FR response rates at the beginning of each session (component period 1) were not effected by the irradiation but that FR responding gradually declined.

The decline in TO responding (Figure 10B) of the irradiated subgroup was immediate and dramatic. Overall TO response rates were decreased by about 75% on the first day and remained at a reduced level throughout the five days of irradiation. Whereas there was some indication that TO responding early in the session (TO component period 1) was less effected, the MWR effect was clearly apparent during that interval. As with the FR response rates, TO responding remained at a reduced level throughout the several weeks of exposure.

When examined in similar detail (not shown), PM-MWR (at a comparable dose rate) had a similar differential effect on the two response components. 3.5 - 3.6 mW/g was close to the behavioral threshold and the results of examination of the PM and CW data on a daily basis are not yet conclusive. Again, there was some suggestion that the decline in TO responding (the major effect at this dose rate) was greater on the first as compared with subsequent days of irradiation. However, there was nothing to indicate that FR responding was transiently reduced to a significant degree.

Differential reinforcement paradigms for MWR behavioral research -

Fixed-ratio operant responding was the logical place to start our studies. This simple format provided a direct verification of the suitability of the technique. Only after this certification was it appropriate to incorporate more complex behavioral paradigms in the project. To have moved to such studies before building a conceptual bridge between this highly specialized apparatus system and traditional, single-subject behavioral protocols and apparatus systems would have imposed needless limitations on the generalizability of these studies.

A "counting" task with characteristics similar to a differential reinforcement of low rate paradigm was felt to be appropriate as well as practical with the single lever instrumentation already in place. The protocol of the counting task (fixed consecutive integer with passive report) was complex but allowed (1) distinct trials procedures with the extant MWR/behavioral apparatus and (2) repeated acquisition format. The latter was viewed as particularly important since it provided a means with which to evaluate cognitive in addition to performance variables.

Upon presentation of the active discriminative stimulus (light on), the task for the animals was to emit a predetermined number of bar presses during the interval that the light was on (S^D, 15 seconds). If correct, a food pellet reinforcement would be delivered at the end of the S^D interval, coincident with the light being extinguished and the end of the trial. The minimum intertrial interval was 10 seconds, but this was contingent upon the animal refraining from bar pressing during the intertrial interval.

In outline, each S^D trial component (signalled by turning on a centrally positioned lamp) could have one of four mutually exclusive results. The animal could either:

(1) not respond,
(2) respond correctly, that is, emit a predetermined number of bar-presses during the trial and wait until the end of trial,
(3) respond with more than the targeted number of bar-presses, or
(4) respond with fewer than the required number of bar-presses.

The only result of responding during the inter-trial period (visual discriminative stimulus lamp turned off, S^d) was a cummulative prolongation of the time-out interval (a penalty, since the potential number of trials during the fixed duration session was reduced thereby). In view of the lengthy training period required for the groups of 30-32 animals to reach criterion on this schedule, each experiment utilizing this format was scheduled for a 7 to 9 month duration. While complex, the format was suitable to the Long-Evans rat (as indicated by the 40-60% success rate on trials) and it allowed studies of repeated acquisition of operant behavior.

The behavioral component of each daily session in the apparatus took 2-1/2 hours. MWR, when applicable, commenced 15 minutes before and terminated 15 minutes after the behavioral session for a total exposure of 3 three hours per session. Each day's performance was divided into 5 serial (continuous) segments of 30 minutes each so as to be able to chart the progressive changes in performance. This "blocking" was in no way detectable by the subjects, for which the behavioral session was one continuous series of trials.

Typically there was a steep fall in TO responding during the early baseline period. By the third week the TO response rates had decreased to an average of one per minute and remained low throughout the remainder of the experiment. The microwave exposure did not commence until the rate of responding had fallen to a stable level.

The first group run on this fixed consecutive integer (FCI) format was exposed to PM MWR at 6.1 mW/g. This was a relatively high dose rate and there developed a prompt reduction in TO responding (figure 11) that was particularly evident upon changing the target value for the trial component. This result was analogous to the that of the mult (FR,TO) experiments. The notations below the abscissa in this and each of the following figures denote "target" count for the FCI trials as well as onset of the MWR. This exposure yielded, however, no clear evidence of an effect on number of correct responses (Figure 12). Several derived measures were calculated. In particular, conditional accuracy (Fig. 13, the probability that an emitted response was correct) nor relative accuracy (Fig. 14, the fractional number of correct responses) revealed any underlying changes in reinforcements. Examination of the data over the course of the sessions showed, retrospectively, a tendency for reduced responding under MWR to develop in the later stages of each session. These observations were confounded, however, by a systematic difference in group performance that preceded MWR exposure and by premature termination of the exposure section of the experiment.

Two additional experiments, likewise using PM-WMR at 6.1 and 3.8 mW/g were undertaken. In each instance the experiment had to be aborted shortly after MWR onset due to intermittent MWR source failure. A second MWR source was obtained and the attempts to repeat these experiment resumed.

To conserve time and improve the effective reliability of the source, a dose rate of 3.7 mW/g was picked for the remainder of the experiments using the FCI format. This dose rate, either PM or CW, was known to correlate with an enhanced rate of extinction of TO responding without coincident effects on FR-25 response rates. It was of considerable interest to establish whether similar results would be obtained in a format where TO responding, while occurring in a similar discriminative stimulus state, had a considerably different operant significance.

A group of 16 control and 15 irradiated animals was run for a two month period on alternating FCI-2, FCI-3 until TO responding had fallen to on the order of one per minute (Fig. 15). After the onset of irradiation (day 21), overall TO responding was reduced. the reduction was much more pronounced during the later phases of each session (not shown) consistent with the results of the mult (FR,TO) experiments). The number of operant reinforcements was less dramatically altered, but over several weeks a relative reduction in performance was apparent (Fig. 16). While small, this difference was quite reliable in view of the manner in which it developed over the course of the daily session coincident with the irradiation. Figure 17-19 trace the operant response rates during the initial, middle and terminal 20% of each daily run. It was apparent that at this dose rate, the effect on the primary operant measure (number of correct responses on a repeated acquisition FCI schedule) was becoming more evident over time.

Whole body temperature changes -

The bulk of the experiments covered by this report yielded behavioral effects at MWR dose rates (either CW or PM) that approached or exceeded a thermogenic threshold. It was important, therefore, to explicitly measure the thermal factors of exposure in the waveguide system. Estimates of the changes in whole body temperature during the course of MWR exposure at dose rates relevant to these experiments were obtained by measuring changes in core (deep rectal) temperature. Two groups of rats, with individual weights falling within the range of the animals used for the behavioral studies, were used. Macroscopic thermal changes should be insensitive to the modulation profile of the MWR; however, to correspond to the behavioral test conditions, separate subgroups were exposed to CW and to PM MWR. Each subgroup was further divided into actual and sham irradiated subgroups.

Each of the sham (control) and irradiated animals was placed into the waveguide immediately after noting its rectal temperature. They remained in the waveguide for either one or three hours. Rectal temperature was then measured once more within ten minutes after removal from the waveguide to establish the net increment due to actual or sham exposure. (Ten minutes was found to be the minimal interval necessary to preclude cool-down errors subsequent to any MWR-induced elevations of body temperature.) The results of these measurements for the one and three hour exposure durations are summarized in Table I.

At dose rates of 3.5 (CW) and 3.4 (PM) mW/g, the approximate behavioral threshold, there were no statistically significant differences in rectal temperature following either the one or three hour exposure. At 6.3 (CW) and 6.4 (PM) mW/g, a significant elevation in whole body temperature of 0.5 to 1.0°C was detected. From the tabulated group data, one might suspect that the CW MWR is the more effective in producing a change in deep rectal temperature. However, the relatively high variability of these data from the CW exposure group at 6.3 mW/g prevents one from taking this possibility seriously at this time. Note that while behaviorally naive animals were utilized, the mean weights and the duration of the exposure for these thermal studies were representative of the conditions pertaining to the animals actually undergoing behavioral study.

Effects of MWR on rate of growth -

All subjects routinely were weighed three times per week throughout their participation in the behavioral studies. Only at the highest dose rate (6.5 mW/g) was there any slight, apparently persistent differences between the growth curves of the control and irradiated groups. In that instance the differences could have represented the continuation of a trend that began before irradiation commenced. A two-factor ANOVA (with repeated measures on weeks) by treatment over the six weeks of irradiation indicated that there was a significant overall weight gain ($F(5, 135)=67.49$, $p<.0001$) but no significant difference between groups in overall weight ($F=.253$) or rate of weight gain ($F=1.59$). Note that although the irradiated groups might show a lowered FR response rate, on the order of 70% of the total food available was provided by the post-session supplement given each rat. The small differences in reinforcements delivered over the course of the constant duration sessions would translate to a 3 to 5% reduction in total food availability for the irradiated group.

TABLE I

A. Mean change in rectal temperature of rats subjected to CW MWR, relative to the temperature just prior to placement in waveguides. Sham-irradiated and irradiated subgroups each consisted of 5 animals (subgroup mean weights: 338 g and 354 g, respectively); animals irradiated for three hours.

SAR		mean T °C(+/-SEM) after one hour	mean T °C(+/-SEM) after three hours
3.5 mW/g	sham-Irr	- .20 (.17)	- .36 (.21)
	Irr	.18 (.18)	.04 (.26)
6.3 mW/g	sham-Irr	.14 (.23)	- .30 (.42)
	Irr	1.06 (.42)[a]	1.12 (.39)[b]

(a) group difference significant at p¼.10 (t-test)

(b) group difference significant at p¼.05 (t-test)

B. Mean change in rectal temperature of rats subjected to PM MWR. Sham-irradiated and irradiated subgroups consisted of 10 and 12 animals, respectively (subgroup mean weights: sham-irradiated, 332 g; irradiated, 342 g).

SAR		mean T °C(+/-SEM) after one hour	mean T °C(+/-SEM) after three hours
3.4 mW/g	sham-Irr	.38 (.16)	.57 (.12)
	Irr	.29 (.15)	.60 (.09)
6.4 mW/g	sham-Irr	.24 (.14)	.42 (.12)
	Irr	.95 (.12)[c]	.92 (.09)[c]

(c) group difference significant at p¼.01 (t-test)

DISCUSSION

The choice of operating frequency (1.3 Ghz) for this exposure system was dictated largely by our intention to study and compare the effects of PM MWR versus CW MWR and, therefore, by the availability of suitable sources at that frequency. The difference between this operating frequency and 918 MHz, where many irradiation studies have already been carried out, is sufficiently small to allow extrapolation and comparison between the two frequencies. However, the smaller cross section of the waveguide used here did imply a tighter coupling between the irradiation and the target than was found by Guy and Chou (1977) in their pioneering studies of waveguide exposure systems for whole animal studies. Our normalized average (whole body) SAR was, for example, approximately 2.2 mW/g for each watt of input power. In the 8 inch waveguide of Guy and Chou, the comparable figure was about 1.2 mW/g per input watt. We also noted a higher ratio of peak to average SAR (7.9 mW/g head versus 2.2 mW/g whole body average, per watt input or a ratio of 3.6 to 1). The comparable figure from Guy and Chou's results was a ratio of 1.63 to 1. These points must be taken into account when attempting to generalize over frequency or from one exposure system to another.

Regional SAR's here were determined using time-temperature methods and found to be consistent with the whole body SAR measured via the multi-port power balance technique. As further verification of the suitability of the chamber for multiport determination of average SAR, there was close agreement between time-temperature and power balance determination of SAR using saline loads of small volume (for rapid thermal equilibration) strategically placed in the waveguide. We estimate that the errors in specification of SAR during behavioral studies did not exceed +,- 10%. However, in using calibration methods to specify the group dose rates, we tended to be conservative in that the specification was such that any accumulated errors would tend to diminish the actual versus the specified dose rate. All dose rate specifications here are, in other words, upper bounds.

A comparative estimate of the "effective" incident power density can be derived, as follows. The distribution of power density over the circular cross section of the waveguide is known from the equations governing power transmission down a circular guide. In the dominant, TE_{11} mode, the radial distribution of the power density can be approximated by a cosine pulse distribution,

$$P(r) = P_p \cos (r/2a)$$

where P_p is the peak power density, r is the radial distance from the center of the circular cross section and a is the radius of the waveguide. Integrating over the cross sectional area of the guide the interior power is therefore

$$P_i = 1.45\ a^2 P_p$$

where P_i is the total power. If the total input power is 1 W, then the peak power density within the waveguide must be 12.2 mW/cm^2. The average power density (total divided by cross sectional area), by the same approximation, would be 5.65 mW/cm^2 for the 15 cm diameter waveguide used here. With a 300 gram rat in place, the measurements of field distribution within the waveguide using a non-invasive probe yielded a peak interior power density of 7 mW/cm^2 per input watt, just in front of the carcass.

Working backwards from data in the Dosimetry Handbook (Durney et al, 1978), an average SAR of 2.2 mW/g per input watt at 1.3 GHZ would imply that the effective incident field density was 9.2 mW/cm^2 per input watt. Actual measurements of average SAR in a rat of that size (Durney et al, 1978) indicated that the nominal 2.2 mW/g figure would correspond to an incident field intensity of 14.2 mW/cm^2 per input watt. From several points of view, therefore, the observed SAR here is, therefore, certainly appropriate to the theoretical distribution of the interior fields.

The majority of the available MWR behavioral research reports on rodents are of limited generalizability because of (a) small population size, (b) behavioral trials that took place after rather than concurrent with the exposure to MWR and/or (c) physical restraint of the test animal. The constraints imposed by the need for a practical, multi-animal exposure system that allows on-line determination of dose rates did not leave much flexibility in the design of the behavioral aspects of such experiments. The circular waveguide array has the capabilities of performing within these constraints and has proven to be an effective tool with which to study the influence of MWR on ongoing operant behavior. Despite some remaining limitations, the unitized MWR and behavioral effects facility offers considerable advantages for examining, with large groups of subjects, MWR interactions with complex behavior, interactions that may develop or be modified over a prolonged period of time.

Operant behavioral experiments of relatively long duration and taking place within the specialized environment of waveguide irradiation chambers have been carried out for the forst time in these studies. The physical condition of the animals remained satisfactory throughout the course of each experiment; being restricted to the irradiation/operant chamber for 3 to 4 hours each day did not interfere with the establishment or maintenance of stable operant performance. Thus, experimental protocols calling for daily operant sessions within the waveguide for periods approaching the normal lifetime of the animals appear to be feasible.

At an average SAR of 1.5 mW/g there was no evidence for an effect on either FR-25 or TO response rates in the simple schedule controlled operant behavior used in the first series of experiments (mult (FR-25,TO 10)). At 3.6 mW/g there was little apparent change in the level of responding for food reinforcement. However, the marked variations in S^d responding demonstrated by the irradiated group during irradiation suggested that there was some significant behavioral interaction throughout the exposure. The variability inherent in the S^d performance rates made this a sensitive but noisy behavioral output parameter. However, on the basis of multiple independent experiments using both CW and PM MWR it is reasonable to conclude that this dose rate was approximately threshold under the multiple component FR, TO format.

At an average SAR of 6.7 mW/g, both FR-25 and TO response rates clearly were affected. FR responding was the more resistant to modification and showed a more rapid rate of satiation but no statistically significant differences in total number of responses per session. This is consistent with the robust schedule control generally observed under FR. The changes in TO responding were much more pronounced. The onset of MWR was accompanied by a prompt reduction in the S^d response rate, especially in the second half of the session. In fact, by the end of the session, most irradiated rats ceased responding entirely, a pattern of behavior that differed markedly from that of all other groups. It was particularly interesting to note that at offset of high MWR dose rates the TO response rates of the irradiated groups showed prompt rebound and subsequent recovery to the level of the control groups. Such rebound is another measure of the behaviorally significant effect of the irradiation and is analogous to the post-irradiation elevation of response rates reported by Mitchell et al. (1977).

It is apparent that the magnitude of the behavioral effects of the MWR exposure were as much dependent upon the behavioral component being studied as upon MWR rate. The relatively robust and tightly controlled FR behavior reinforced by food was in contrast to the more labile and variable bar pressing rates during S^d. Thus, the extent of schedule control clearly is a critical parameter in establishing the relevance of effective microwave "thresholds". Unfortunately, the data did not give a direction indication of the nature of the cue or of the internal stimulus by which the behavioral changes were instigated. In view of the high dose rate at which the most clear cut changes were observed and of the similar nature of the effects on S^d responding at high versus threshold dose rates, thermal factors are very likely to have been involved. Further, the rebound in TO responding subsequent to termination of the MWR suggests that a non-neutral cue was involved.

With PM MWR, microwave hearing (Frey, 1967; Lebovitz and Seaman, 1977) is a potential source of behavioral cues. The magnitude of the intracranial acoustic cue has been verified using studies of single unit activity (Lebovitz and Seaman, 1977), evoked potentials (Taylor and Ashleman, 1974) as well as behavioral endpoints (Frey and Feld, 1975). Taking the highest dose rate used here and assuming that the intracranial dose rate is as much as three times the whole-body rate, the energy deposition per pulse for a 1 microsecond pulse at 600 pps, would be

$$3 \times 6.7 \times 10^{-3} \;/\; (600) \;=\; 33.5 \times 10^{-6} \;\; \mathrm{J/g.}$$

This energy dose is above the threshold for auditory single units (Lebovitz and Seaman, 1977) and human perception (Guy et al, 1975). It is not clear, however, that the perceived loudness (the "effective acoustic equivalent", Lebovitz and Seaman, 1977) of the 1 microsecond pulse would be adequate to account for the observed changes in behavior. Furthermore, if an acoustic cue were the basis for the above findings, then it would not be immediately obvious why the major reduction in S^D operant responding should develop gradually rather than immediately upon onset of the irradiation. The studies utilizing CW MWR have yielded essentially the same findings as did PM MWR. It would seem necessary to discount auditory cues as the basis for the results reported above. In fact a major aspect of the later stages of this work was to determine whether CW MWR would yield any substantially different effects on operant responding than did PM MWR and thus provide evidence contradictory to a simple thermal basis for such effects.

Based upon the form of the observed modifications in FR and TO rates of responding, 1.3 GHz CW irradiation yielded, however, essentially the same findings as did PM MWR. It is worth emphasizing that even with dose rates sufficient to yield a 0.5 to 1.0^{o}C elevation in rectal temperature, FR performance was only marginally reduced. At 3.6 mW/g, the behavioral as well as the thermogenic threshold, a MWR induced decline in TO responding was evident and not accompanied by a corresponding reduction in FR responding. The suppression (enhanced stimulus control under this format) of TO responding was consistent with the demonstrated reduction in level of motor activity of rodents exposed to moderate to high levels of MWR (Sanza and de LOrge, 1977). These results suggested that more precisely formulated studies, utilizing a behavioral format with a greater degree of sensitivity and temporal discrimination, were required (see below).

The gradual and initially evanescent decline in FR responding, in response to CW MWR at a moderately high dose rate of 5.9 mW/g closely coincided with the results of experiments using PM MWR exclusively. Those studies showed that only after two to three weeks was the full effect of an SAR of 6.7 mW/g on FR responding observed. On the other hand, the immediate decline in TO responding was essentially the same as that found after exposure to PM MWR at a comparable dose rate. In virtually all respects, therefore, the differential effect of MWR on the FR and TO components of this multiple schedule are the same with CW as with PM MWR.

Finding equivalent effects of PM and CW MWR at comparable dose rates is frequently taken as support for, if not a verification of, an essentially thermal basis for the underlying interaction. Indeed, purely thermal mechanisms would require such an equivalence. While the data here are consistent with a thermal basis, they cannot be extended to imply that all behavioral effects of MWR are based on steady state thermal changes.

Sensitivity to thermal cues has been demonstrated in monkeys at MWR dose rates equivalent to only a fraction of the resting metabolic rate (Adair and Adams, 1980). Furthermore, it has been shown that when chilled, rats will emit instrumental responses to gain MWR (Stern et al., 1979). The experiments presented here were carried out at normal ambient temperatures; however, the added thermal load could easily have been sufficient to condition motor activity. Certainly the internal distribution of the absorbed energy would be dependent upon changes in orientation of the animal with respect to the launching (behavioral) end of the waveguide (Guy and Chou, 1977). When facing the behavioral apparatus, the peak energy absorption will be in the head of the target animal. When facing away from the behavioral apparatus, the peak will occur in the hindquarters. The reduced likelihood of the animal to bar press during S^{d}, assuming that this did not represent a diminished capacity to do so (viz., TO response rates were only marginally diminished), therefore may be explained as the result of an attempt to redistribute the imposed thermal load by turning away from the operandum. It is important to recognize, however, how much less likely the irradiated animals were to orient away from the operant area when food reinforcement was available.

The resting metabolic rate for rats is 4.9 mW/g$^{(0.75)}$ (Durney et al., 1978) or 7.0 mW/g for a 240 gram rat. Thus, MWR at 6.7 mW/g represents a virtual doubling of the heat dissipation requirements and, therefore, is certainly a thermally significant endogenous heat load. Simple exercise, on the other hand, may raise the metabolic rate by a factor of 5 to 10 above the resting level without deleterious effects (Nodel et al., 1977). Gage et al (1979) have reported that rats do not orient relative to the E-field of thermally significant MWR. However, in their experiments the energy was incident upon the dorsal surface of the animal (animal longitudinal axis in the plane of the E-field). Here, the E-field was orthogonal to the longitudinal axis of the waveguide and, generally speaking, in a plane parallel to the dorsoventral axis of the irradiated animals. It remains to be determined whether shifts in posture/orientation and changes in activity levels during irradiation will account directly for the observed changes in operant response rates. There was little in these data to suggest that the animals' motor or discriminative capabilities were impaired by exposure to the MWR. The reduced rate of TO responding could then be looked upon as deriving either directly from a modification in the capability or willingness of the animal to bar-press coincident with the slight thermal cues, or perhaps indirectly as a result of the animals' adoption of behavioral strategies to redistribute the imposed thermal load. Whereas the latter would appear to be consistent with the animals' capabilities, at least one study has shown that rats do not necessarily orient themselves with respect to the thermally significant incident MWR energy (Gage et al, 1979). Unless a precise locus of interaction can be postulated, the specification of MWR dose in terms of whole body average absorbed-dose rate offers the only readily communicated and meaningful parameter. Obviously, a chief aim of future work should be to specify more precisely both site and details of the critical interactions.

The complex FCI format (here implemented on a single lever using passive report)) provides a means for more detailed studies of the temporal distribution as well as frequency of operant responding. Only limited entry into this area had been achieved before the end of the project and the data will require more complete analysis before they can be fully discussed. While not explicable in all their detail, the results to date confirm our previous findings regarding a differential effect of MWR at 3-4 mW/g on particular behavioral components. The significant reduction in the rate of acquisition and reacquisition when exposed to MWR at 3.2 mW/g both extended our earlier findings of a significant behavioral effect of that level and indicated that, in the appropriate behavioral setting, more significant behavior that simply incidental (i.e., TO response rates) would be effected. A response having definite survival value for the animal (reinforced, low rate bar pressing) was markedly retarded, in comparison both with the parallel controls and with the behavior of the same animals during prior, non-irradiation components of the experiment. Experiments at lower dose rates have not been completed.

Exposure to microwave radiation poses some unusual and critical problems of safety since we are largely insensible to this form of energy. Especially at low dose rates, we derive few conscious cues with which to develop effective escape strategies, should these be appropriate. While imperfect if carried to extreme, the analogy between MWR and particulate radiation helps to illustrate several basic points. First, damage or significant biological changes may be incurred without alerting the target organism. of sensitivity to the absorbed energy. Thirdly, such damage as may result may not be given expression until much later in the lifetime of the exposed organism. Neither our basic knowledge of MWR interaction mechanisms nor our ability to specify the critical modes of interaction underlying behavioral modulation is sufficient to satisfy all investigators that we can make readily defensible deductions regarding the results of exposure to low levels of MWR.

It has been proposed for some time, that the complexity of organization of the central nervous system makes it susceptible to subtle MWR interactions that may not be seen easily in the unorganized properties of tissue or tissue surrogates. Numerous studies have provided evidence that complex behavioral alterations may result from exposure to levels of irradiation below that which yields measurable elevations in whole body temperature. These include alteration in attention states and motor activity, modification of performance and accuracy in instrumental conditioning paradigms, modification of higher cognitive functioning such as rate and efficiency of learning operant tasks, and altered thermoregulatory functions. The question is whether such results require an explanation other than joule absorption (heating) or rather that the thermal interaction is only obscured, as by the concurrent operation of physiological heat loss mechanisms or appropriate behavioral strategy. The possibility remains of biological interactions that do not derive from even localized temperature changes or thermal load in the usual sense (for example, protein reorientation, resonance absorption and conformational change in complex biomolecules, dielectric interactions, and so forth). It is therefore of considerable current interest to decide whether we must look to such macroscopically athermal mechanisms to explain the observed effects of MWR on animal behavior.

Another factor in the study of MWR biological effects is time. That is, it is proposed increasingly that certain effects may not appear immediately but rather emerge over the exposure history of the test organism. In the case of behavioral effects, such data would suggest that MWR cues or central effects accumulate over time even though the significance of isolated exposure may be minimal and imperceptible. Our experiments have therefore included prolonged protocols with long intervals of irradiation to probe questions of latency as well as concurrency. The results would tend to indicate that MWR induced behavioral effects are maximal at the onset and, if anything, we tend to see behavioral accommodation over time. Further detailed investigation using more sensitive behavioral tasks and measures will need to be carried out in order to support this impression.

The following general points should be noted:

(1) - It was apparent that behavioral alterations by MWR were as much dependent upon the behavioral component being studied as upon the MWR dose rates. FR behavior mediated by prompt food reward was relatively robust and tightly controlled as compared with non-cued bar pressing rates. The extent of stimulus control, in other words, was a critical parameter in establishing the relevance and significance of effective microwave "thresholds".

(2) - In view of the SAR at which the most clear cut changes were observed, thermal factors are a likely basis. Further, the rebound in S^d responding subsequent to termination of the MWR suggests that a non-neutral cue may have been involved.

(3) - Another basis for a significant cue from PM_d MWR is microwave hearing. However, the slow onset of the reduction in S^d responding and the similar results in studies using continuous wave MWR (manuscript in preparation) would tend to discount an auditory cue as critical to the observed changes in operant behavior.

(4) - Motor activity data were not formally collected for these animals. However, reduced motor activity during irradiation and rebound after was a common observation at dose rates in excess of 4 mW/g.

(5) - Such behavioral changes as have been observed to date are most consistent with the hypothesis of weak thermal cues and are readily reversible following offset of irradiation. However, the behavioral strategies adopted in face of even mild thermal challenge can modify ongoing and post-irradiation behavior patterns.

(6) Below the 3 mW/g threshold, no alterations in bulk operant performance has been found, even with exposure durations up to eleven weeks. More sensitive behavioral measures, such a temporal distribution, may yet reveal effects at the lower levels. The interpretation of such effects must, therefore, take into account the depth of study required to detect them.

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FIGURE LEGENDS

FIGURE 1. Cued FR-25 (upper) and TO operant responding of irradiated (N = 15) and sham irradiated (N = 15) rats at an SAR (PM-MWR) of 1.5 mW/g, under a repeated multiple schedule - (MULT(FR-25,TO-10))6. The abscissa indicates the experimental period by weeks; the pre- and post-irradiation control periods are explained in text. The ordinate shows the mean daily number of ratios completed or number of TO responses over the entire session (A, upper and lower). Panels B-D shows corresponding data for session components (blocks) 1, 3 and 6.

FIGURE 2. Operant responding by irradiated (N = 14) and sham irradiated (N = 15) rats at an SAR (PM-MWR) of 3.6 mW/g. Indicated are the mean daily number of ratios completed (upper) and TO responses (lower) over the entire session (panels A) and for component periods (blocks) 1, 3 and 6 (panels B,C and D, respectively), as in Fig. 1. The asterisks denote that simple pair-wise comparisons yielded a statistically significant difference (* - p¼.05; ** - p¼.01) between the means for control and irradiated groups, via a non-parametric (Mann-Whitney U) test.

FIGURE 3. Operant responding by irradiated (N = 15) and sham irradiated (N = 15) rats at an SAR (PM-MWR) of 6.7 mW/g. Shown are the mean daily number of ratios completed (upper) and TO responses (lower) over the entire session (panels A) and for component periods (blocks) 1, 3 and 6 (panels B,C and D, respectively), as in Fig. 1. The asterisks denote that simple pair-wise comparisons yielded a statistically significant difference (* - p¼.05; ** - p¼.01) between the means for control and irradiated groups, via a non-parametric (Mann-Whitney U) test.

FIGURE 4 - Operant responding (6(FR(2515, TO-10) of rats (15 control, 15 irradiated) exposed to CW MWR at an SAR of 5.9 mW/g. Shown are the mean rates of bar-pressing (A.) - per FR component period and (B.) - per TO component period, for the week preceding irradiation and the week of irradiation. The following notations indicate the results of non-parametric statistical tests of the significance of differences between specific pairs of mean rates: (1) difference between treatment subgroup means was significant at the level p¼.02 using a Mann-Whitney U-test, and (2) difference between pre-MWR and MWR weekly means by subgroup (i.e., by subject) was significant at the level p¼.02 using a Wilcoxon signed-ranks test. Full statistical treatment (ANOVA) discussed in text.

FIGURE 5 - Operant responding (6(FR(2515, TO-10) of rats (15 control, 15 irradiated) exposed to CW MWR at an SAR of 3.6 mW/g. Shown are the mean rates of bar-pressing (A.) - per FR component period and (B.) - per TO component period, for the week preceding irradiation and the week of irradiation. The following notations indicate the results of non-parametric statistical tests of the significance of differences between specific pairs of mean rates: (1) difference between treatment subgroup means was significant at the level p¼.02 using a Mann-Whitney U-test, and (2) difference between pre-MWR and MWR weekly means by subgroup (i.e., by subject) was significant at the level p¼.02 using a Wilcoxon signed-ranks test. Full

statistical treatment (ANOVA) discussed in text.

FIGURE 6 - Summary of the changes in FR-25 response rates during a week of irradiation relative to baseline, as a function of FR component period. Each irradiated subgroup (open symbols) can be paired with its parallel-run controls (closed symbols). PM MWR with: SAR 1 = 1.5 mW/g; SAR 2 = 3.6 mW/g; SAR 3 = 6.7 mW/g.

FIGURE 7 - Summary of the changes in FR-25 response rates during a week of irradiation relative to baseline, as a function of FR component period. Each irradiated subgroup (open symbols) can be paired with its parallel-run controls (closed symbols). CW MWR with: SAR 1 = 1.9 mW/g; SAR 2 = 3.6 mW/g; SAR 3 = 5.9 mW/g.

FIGURE 8 - Summary of the changes in TO response rates during a week of irradiation relative to baseline, as a function of FR component period. Each irradiated subgroup (open symbols) can be paired with its parallel-run controls (closed symbols). PM MWR with: SAR 1 = 1.5 mW/g; SAR 2 = 3.6 mW/g; SAR 3 = 6.7 mW/g.

FIGURE 9 - Summary of the changes in TO response rates during a week of irradiation relative to baseline, as a function of FR component period. Each irradiated subgroup (open symbols) can be paired with its parallel-run controls (closed symbols). CW MWR with: SAR 1 = 1.9 mW/g; SAR 2 = 3.6 mW/g; SAR 3 = 5.9 mW/g.

FIGURE 10 - Daily FR (A) and TO (B) response rates during selected portions of daily sessions, to present details of the differentiation between the onset of changes in FR and TO responding. Operant responding (6(FR(2515, TO-10) of rats (15 control, 15 irradiated) exposed to CW MWR at an SAR of 5.9 mW/g on days 6 through 10.

FIGURE 11 - Computer generated plot of mean (+,- SE of the mean) bar-pressing of sham (control) and irradiated (experimental) rats during time-out. Shown are the total non-cued (time-out or error) responses for each daily session of a 50 day experimental run (not including weekends, when animals were not run). The notations below abscissa are discussed in text. MWR onset (PM, 6.1 mW/g) at day 14.

FIGURE 12 - As in Fig. 11, here showing the operant rewards received as a function of session day. The sharp declines and slow recovery coincide with the alternation in trial "target" and give rise to biweekly learning curves.

FIGURE 13 - As in Fig 11, here showing the relative trial efficiency (x100), which is defined as the ratio: (operant rewards received)/(trials). These ratios are computed for each rat; shown are the means and SE of the mean by group.

FIGURE 14 - As in Fig. 13, here showing the relative conditional accuracy (x100) which is defined as the ratio: (operant rewards received)/(number of trials in which some operant response was made).

FIGURE 15 - Total session intertrial (TO) responding during alternating FCI-2, FCI-3 in a group pf 16 control and 15 irradiated rats. The numbers below the abscissa indicate the start of each FCI segment. MWR (PM MWR at 3.7 mW/g)

commenced on day 21 relative to the start of the baseline phase.

FIGURE 16 - Total operant reinforcements per session during alternating FCI-2, FCI-3 in a group pf 16 control and 15 irradiated rats. The numbers below the abscissa indicate the start of each FCI segment. MWR (PM MWR at 3.7 mW/g) commenced on day 21 relative to the start of the baseline phase.

FIGURE 17 - Operant reinforcements during first 20% of session during alternating FCI-2, FCI-3 in a group pf 16 control and 15 irradiated rats. The numbers below the abscissa indicate the start of each FCI segment. MWR (PM MWR at 3.7 mW/g) commenced on day 21 relative to the start of the baseline phase.

FIGURE 18 - Operant reinforcements during middle 20% of session during alternating FCI-2, FCI-3 in a group pf 16 control and 15 irradiated rats. The numbers below the abscissa indicate the start of each FCI segment. MWR (PM MWR at 3.7 mW/g) commenced on day 21 relative to the start of the baseline phase.

FIGURE 19 - Operant reinforcements during terminal 20% of session during alternating FCI-2, FCI-3 in a group pf 16 control and 15 irradiated rats. The numbers below the abscissa indicate the start of each FCI segment. MWR (PM MWR at 3.7 mW/g) commenced on day 21 relative to the start of the baseline phase.

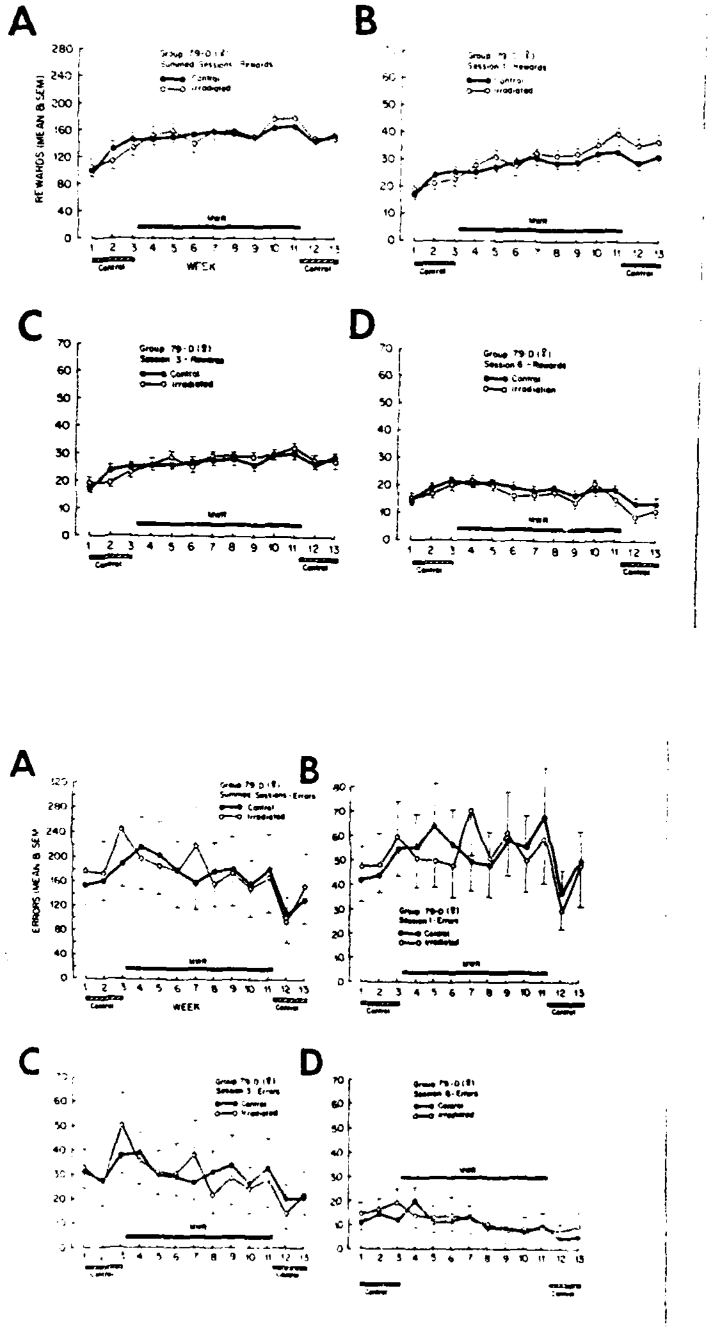


FIGURE 1

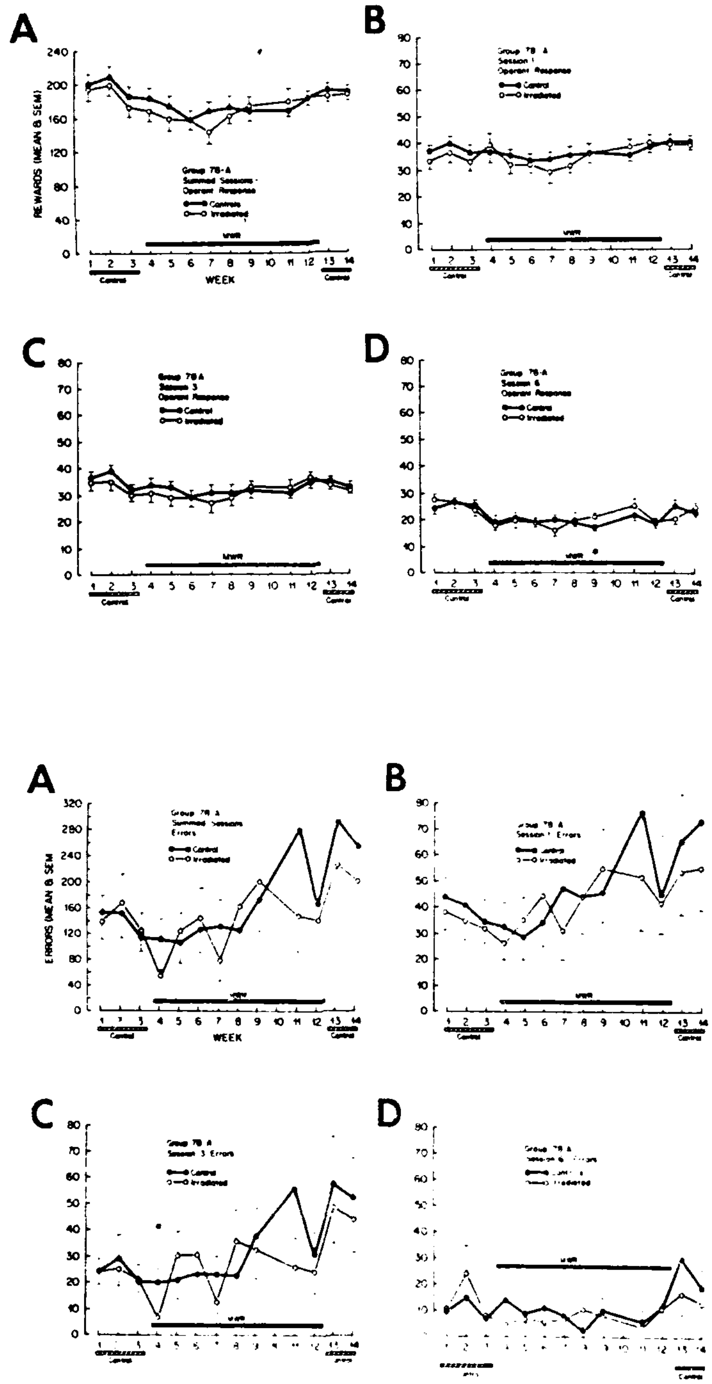

A
B
C
D
REWARDS (MEAN & SEM)
WEEK
ERRORS (MEAN & SEM)
Control
Irradiated

FIGURE 2

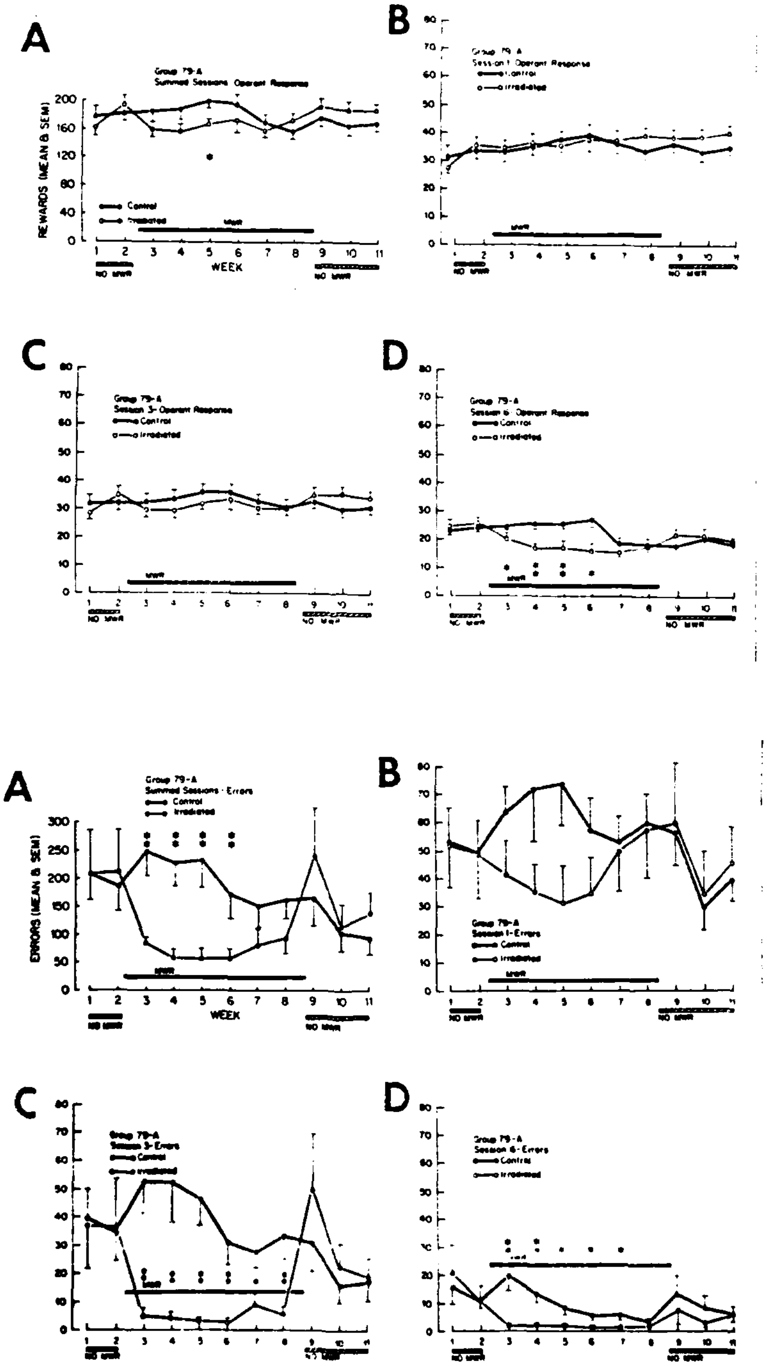

A
B
C
D
Group 79-A
Summed Sessions Operant Response
REWARDS (MEAN & SEM)
Control
Irradiated
MWR
NO MWR
WEEK
A
B
C
D
Group 79-A
Summed Sessions - Errors
ERRORS (MEAN & SEM)
Control
Irradiated
WEEK

FIGURE 5

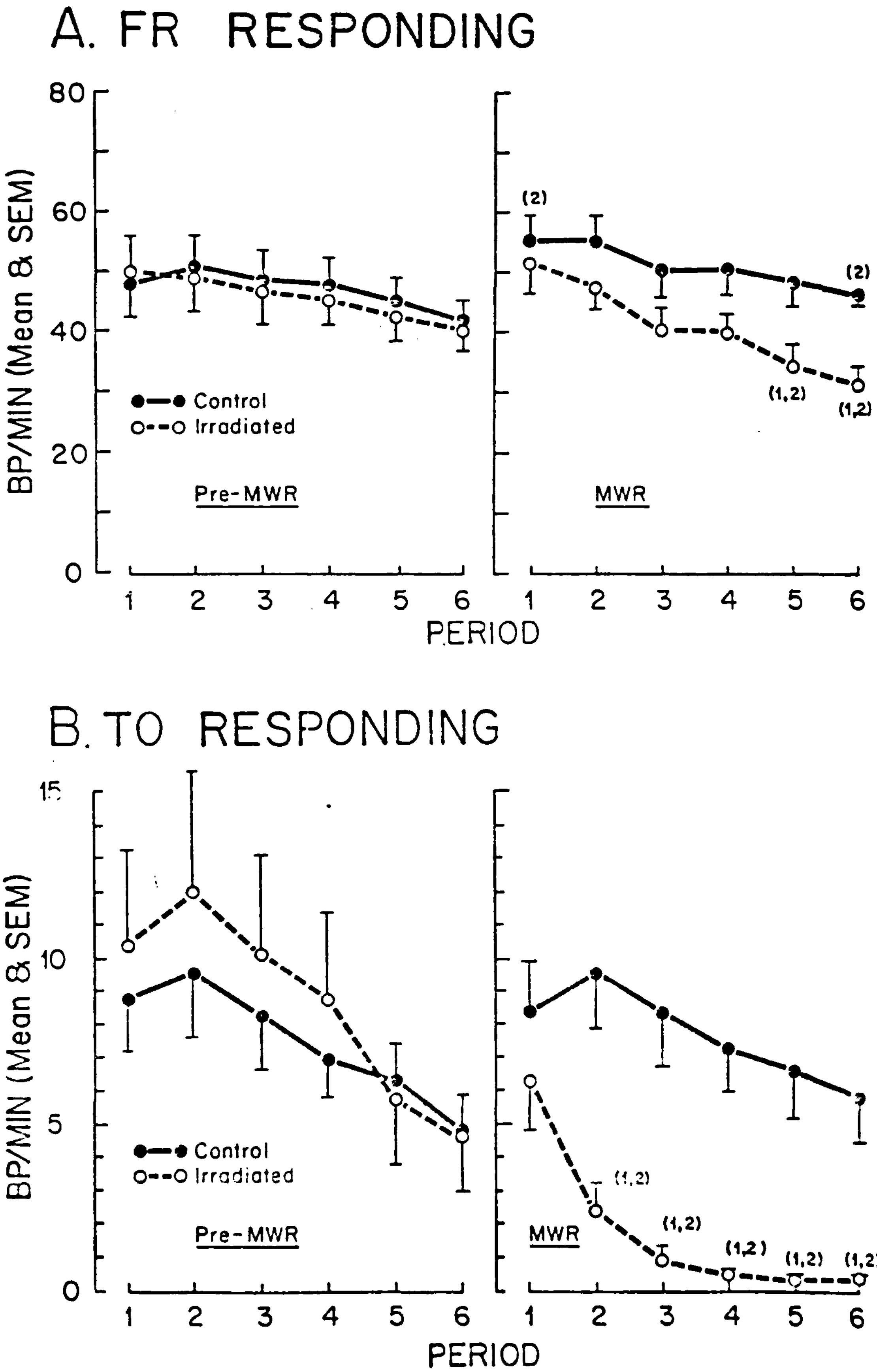

A. FR RESPONDING
80
60
40
20
0
BP/MIN (Mean & SEM)
(2)
(2)
(1,2)
(1,2)
Control
Irradiated
Pre-MWR
MWR
1
2
3
4
5
6
PERIOD
B. TO RESPONDING
15
10
5
BP/MIN (Mean & SEM)
Control
Irradiated
Pre-MWR
MWR
(1,2)
(1,2)
(1,2)
(1,2)
(1,2)
PERIOD

FIGURE 4

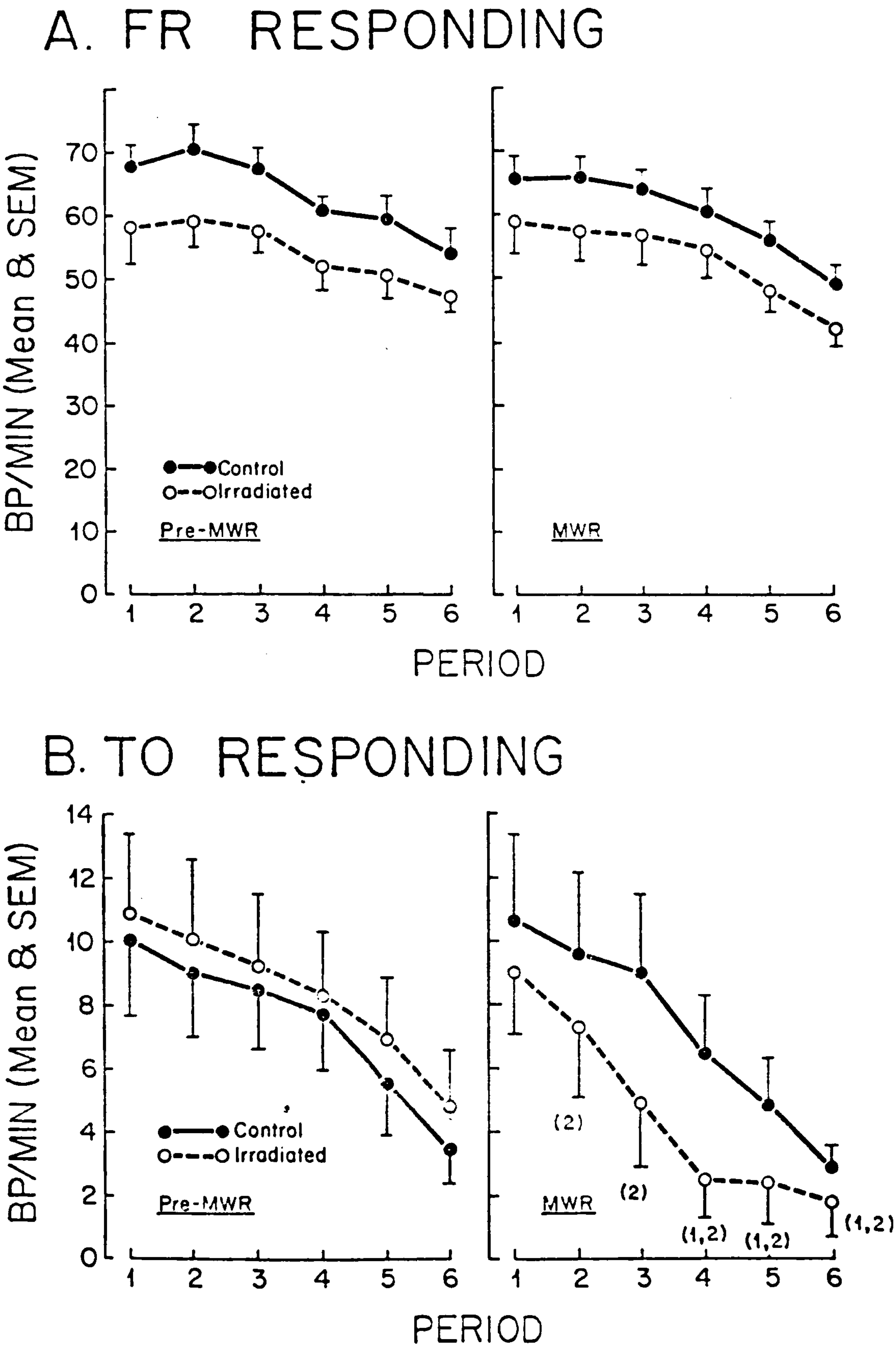

A. FR RESPONDING
BP/MIN (Mean & SEM)
0
10
20
30
40
50
60
70
Control
Irradiated
Pre-MWR
MWR
1
2
3
4
5
6
PERIOD
B. TO RESPONDING
BP/MIN (Mean & SEM)
0
2
4
6
8
10
12
14
Control
Irradiated
Pre-MWR
MWR
(2)
(2)
(1,2)
(1,2)
(1,2)
PERIOD

FIGURE 5

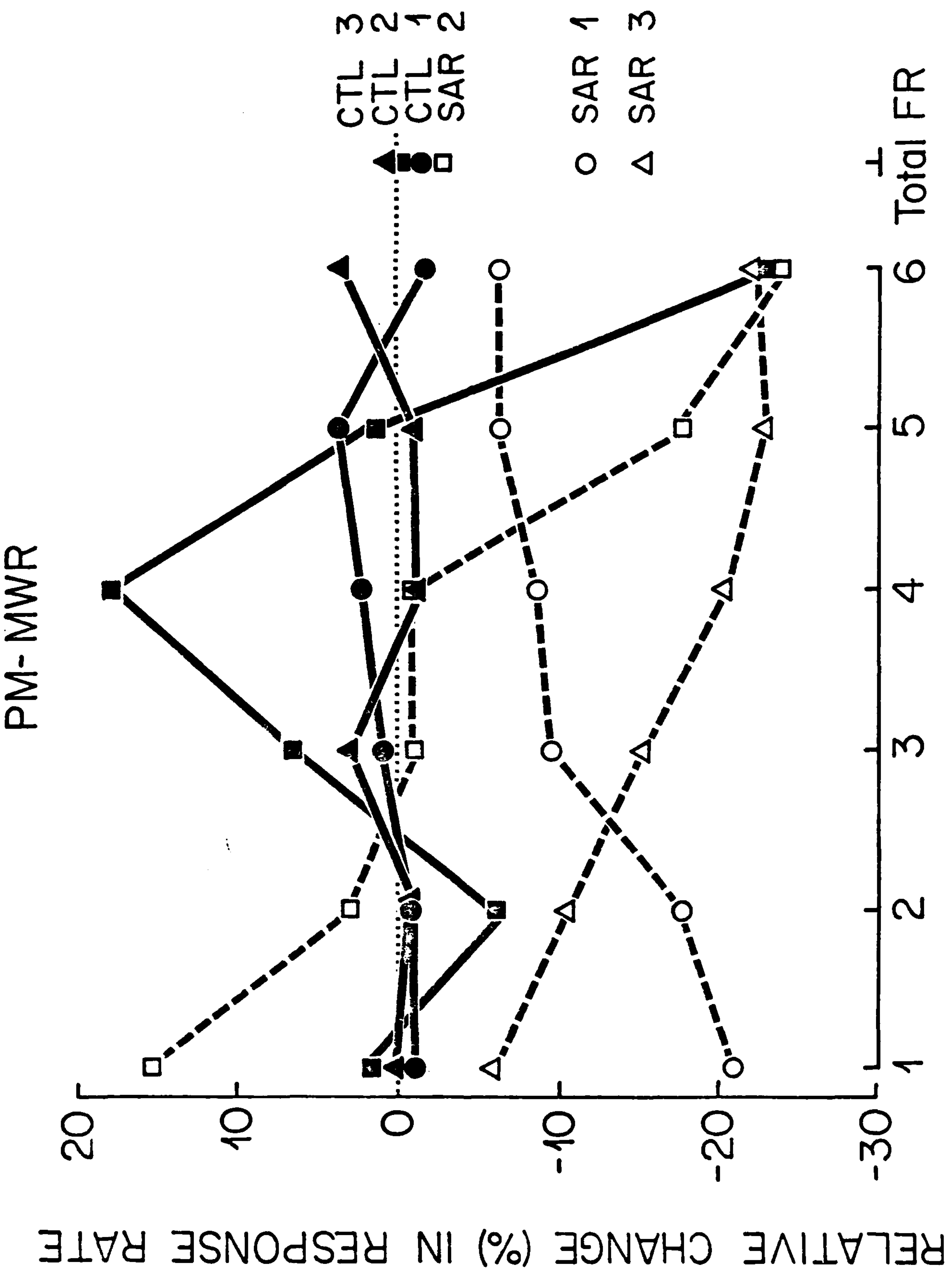
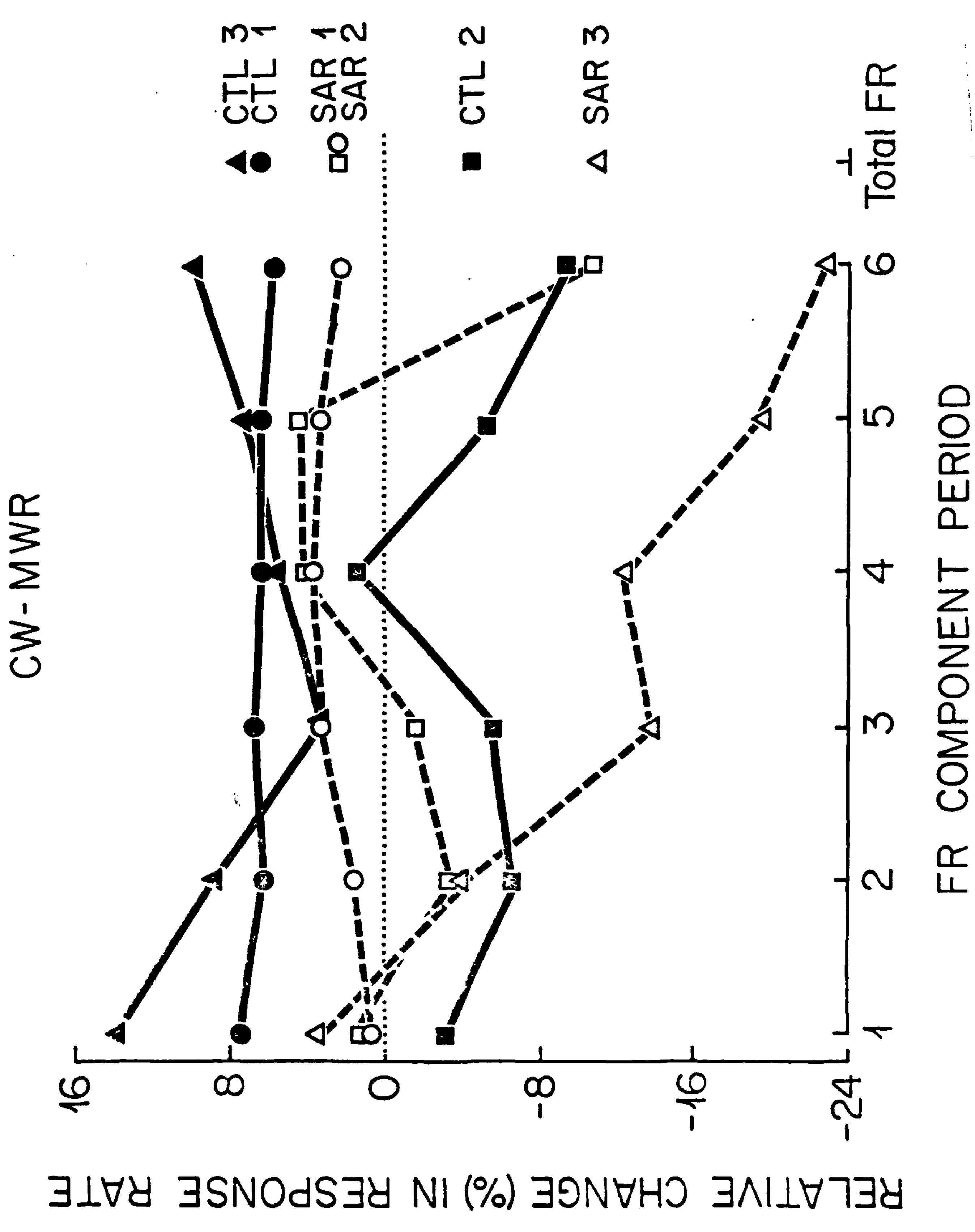

PM-MWR
RELATIVE CHANGE (%) IN RESPONSE RATE
20
10
0
-10
-20
-30
FR COMPONENT PERIOD
1
2
3
4
5
6
Total FR
CTL 3
CTL 2
CTL 1
SAR 2
SAR 1
SAR 3

FIGURE 6



CW-MWR
CTL 3
CTL 1
SAR 1
SAR 2
CTL 2
SAR 3
Total FR
FR COMPONENT PERIOD
1
2
3
4
5
6
RELATIVE CHANGE (%) IN RESPONSE RATE
16
8
0
-8
-16
-24

FIGURE 7

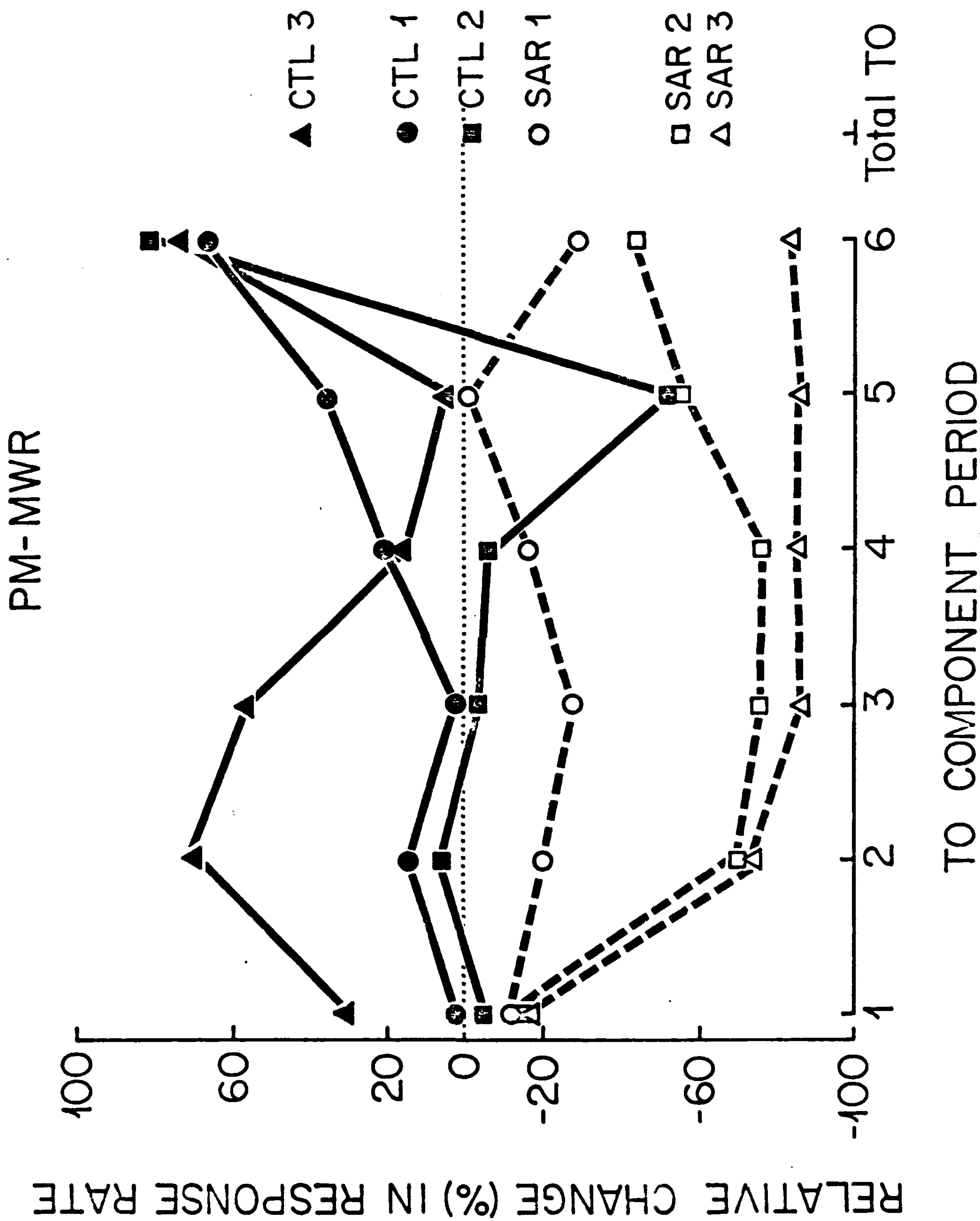

PM-MWR
▲ CTL 3
● CTL 1
■ CTL 2
○ SAR 1
□ SAR 2
△ SAR 3
Total TO
RELATIVE CHANGE (%) IN RESPONSE RATE
100
60
20
0
-20
-60
-100
1
2
3
4
5
6
TO COMPONENT PERIOD

FIGURE 8

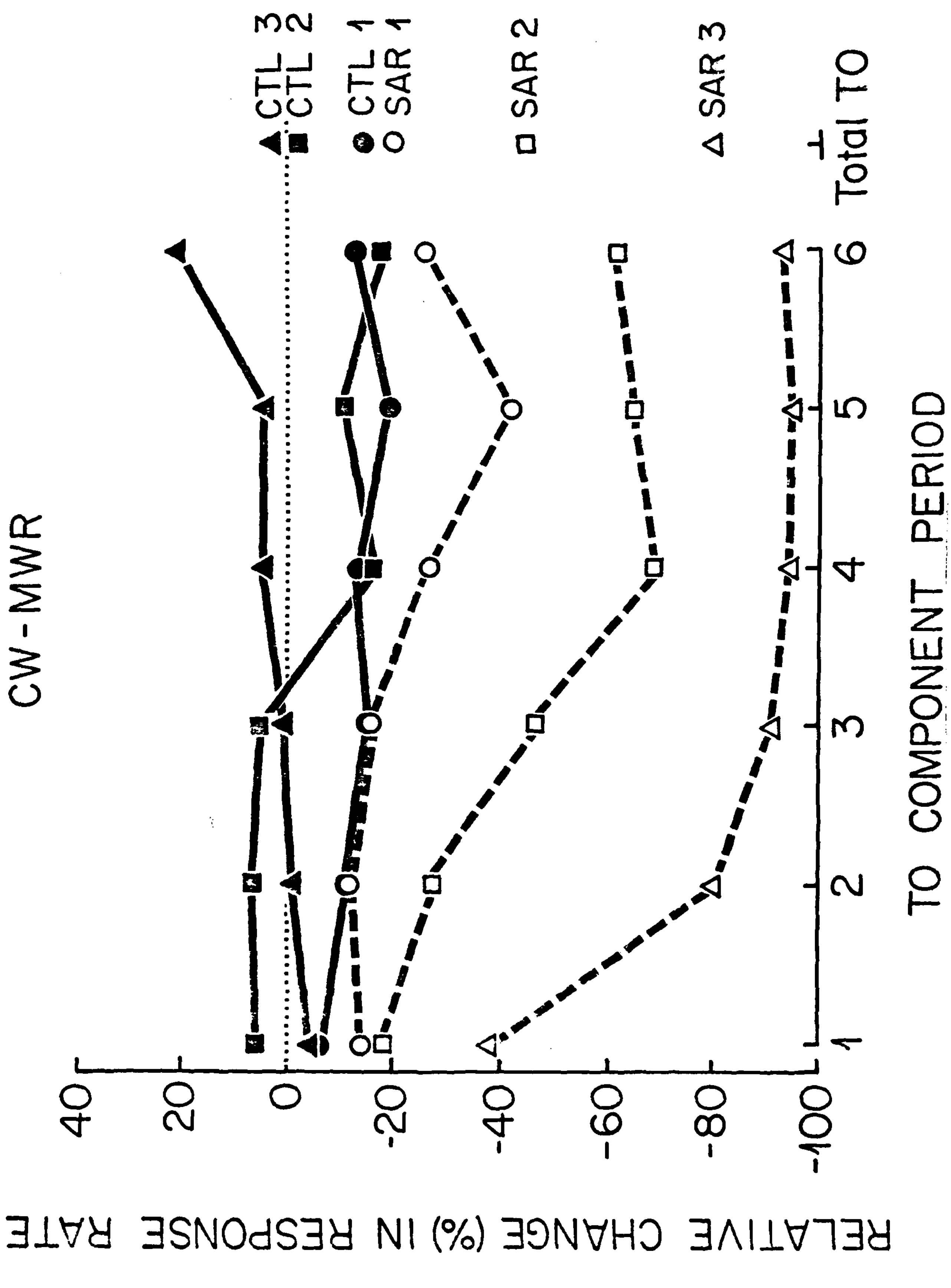
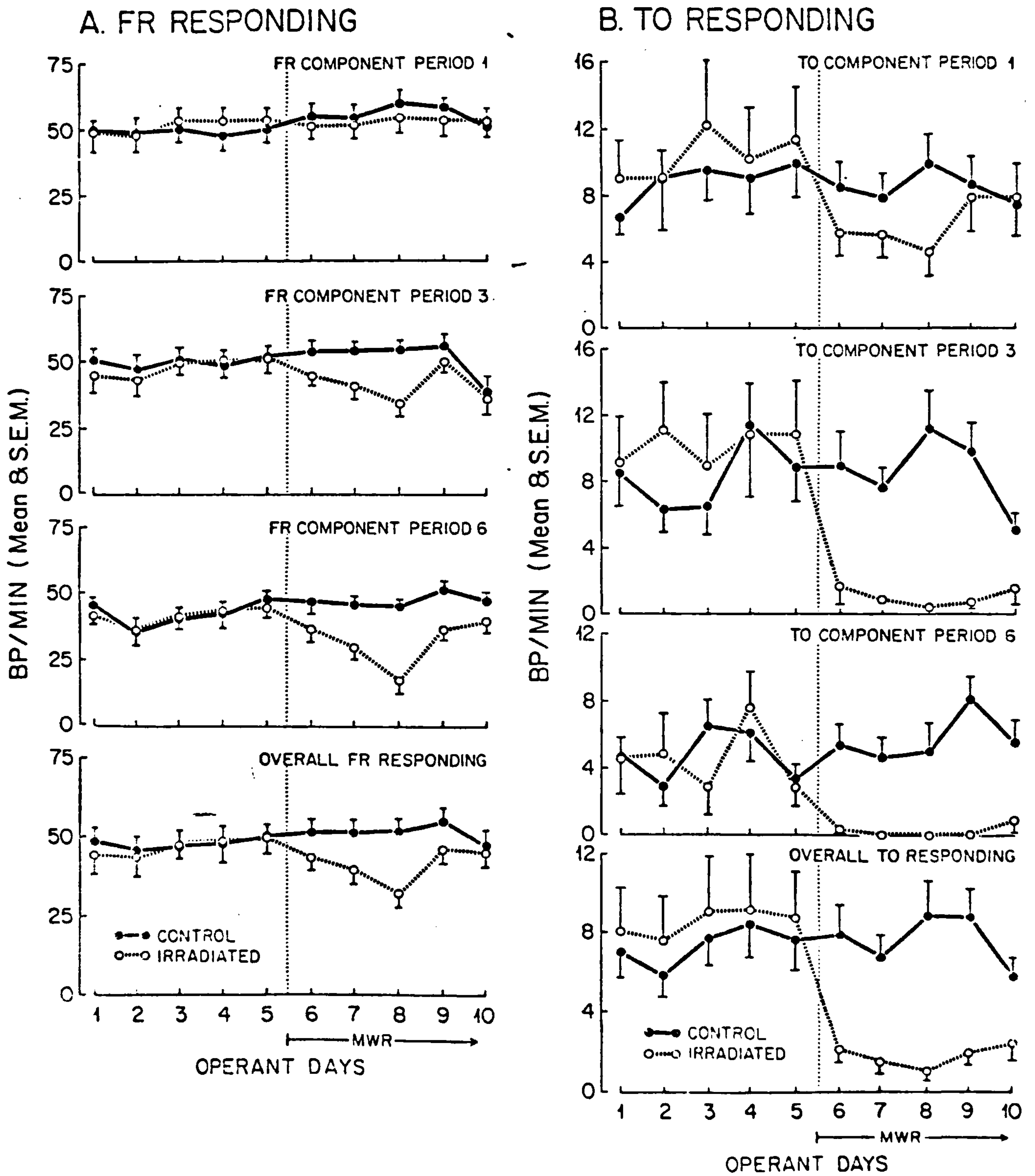

CW-MWR
▲ CTL 3
■ CTL 2
● CTL 1
○ SAR 1
□ SAR 2
Δ SAR 3
Total TO
40
20
0
-20
-40
-60
-80
-100
1
2
3
4
5
6
TO COMPONENT PERIOD
RELATIVE CHANGE (%) IN RESPONSE RATE

FIGURE 9



A. FR RESPONDING
B. TO RESPONDING
FR COMPONENT PERIOD 1
FR COMPONENT PERIOD 3
FR COMPONENT PERIOD 6
OVERALL FR RESPONDING
TO COMPONENT PERIOD 1
TO COMPONENT PERIOD 3
TO COMPONENT PERIOD 6
OVERALL TO RESPONDING
BP/MIN (Mean & S.E.M.)
CONTROL
IRRADIATED
MWR
OPERANT DAYS

FIGURE 10

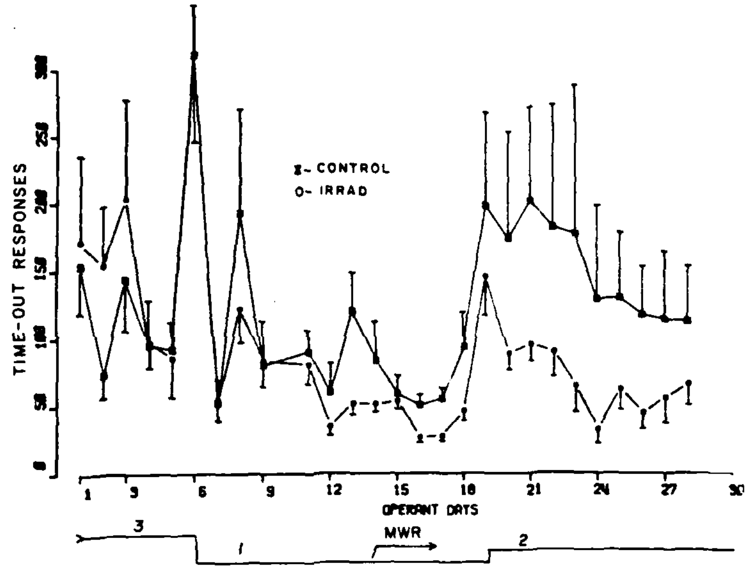


FIGURE 11

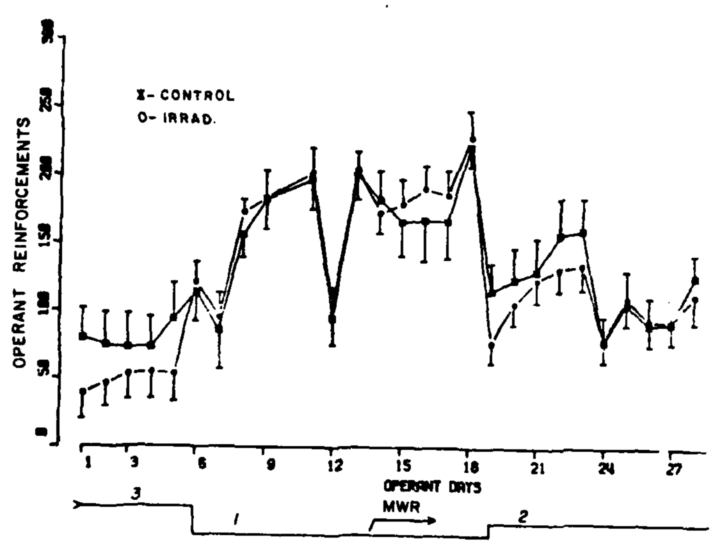


FIGURE 12

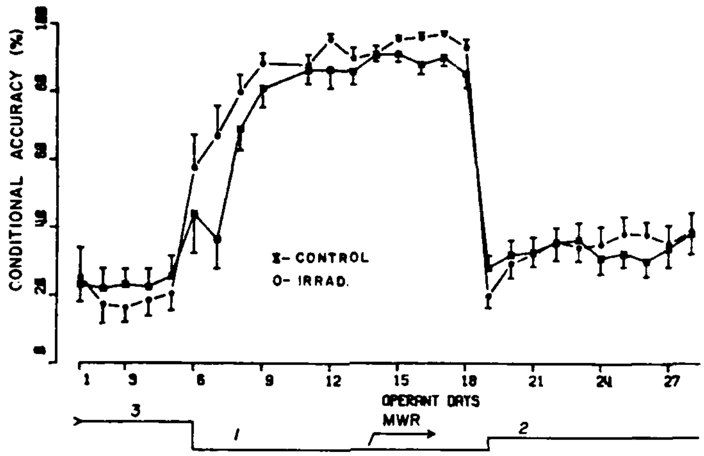


FIGURE 13

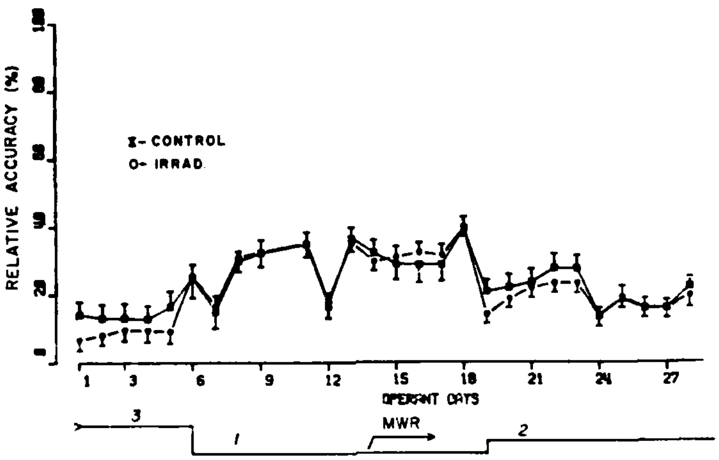


FIGURE 14

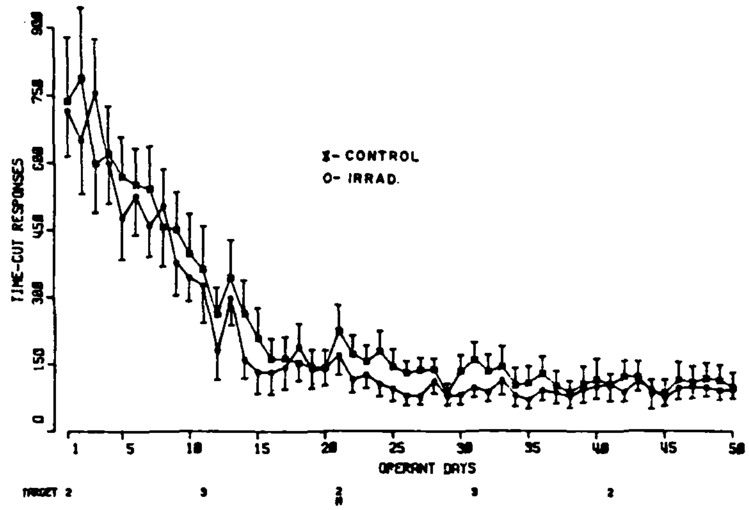


FIGURE 15

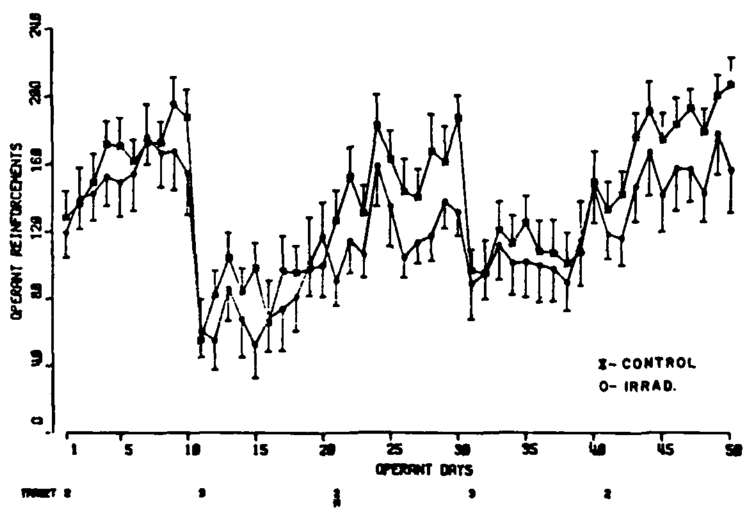


FIGURE 16

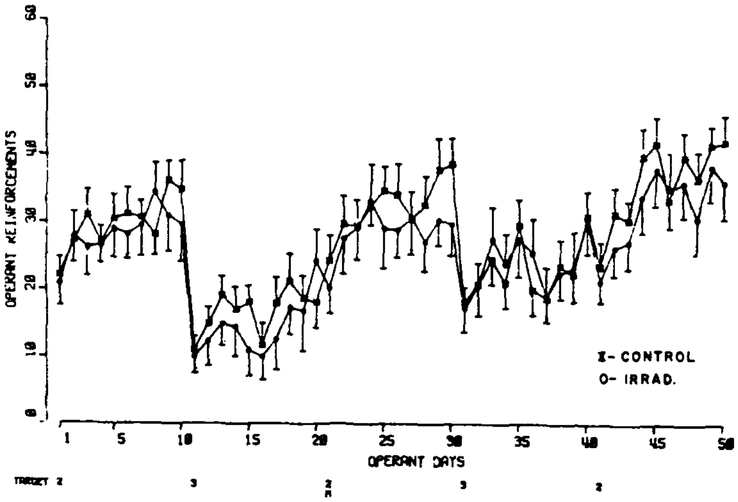


FIGURE 17

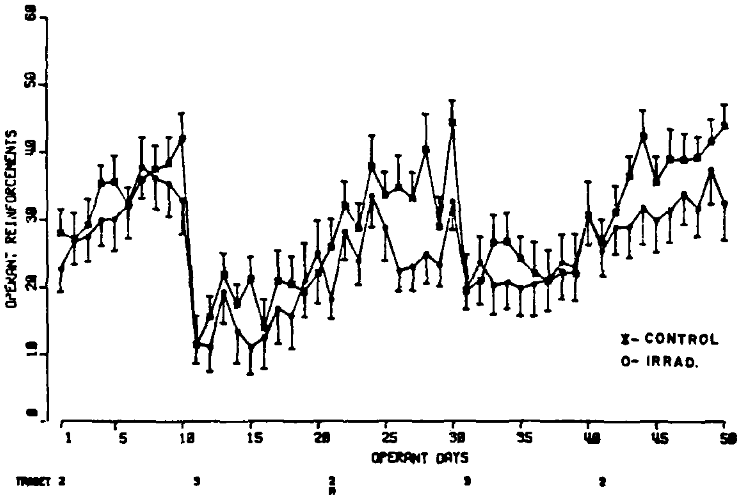


FIGURE 18

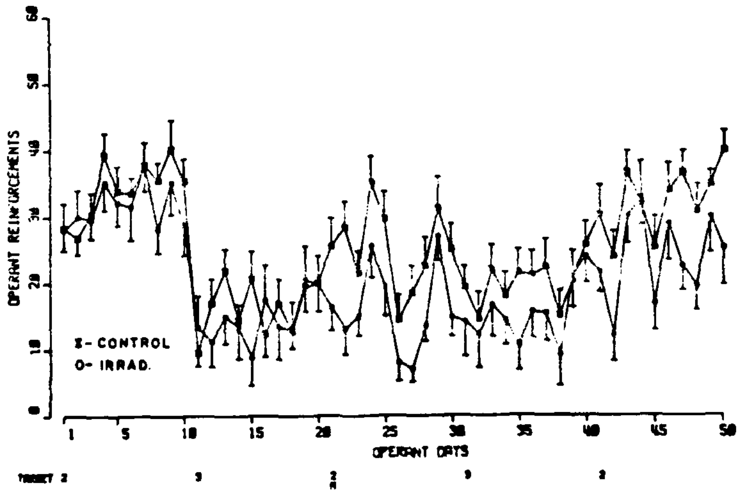


FIGURE 19

APPENDIX
PUBLICATIONS

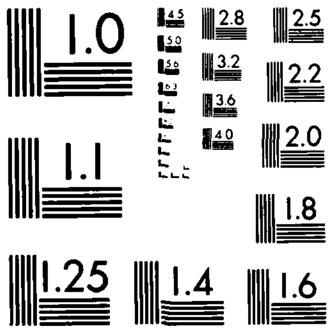
Lebovitz, R.M. Behavioral changes during long-term microwave irradiation. Proceedings Int. Symp. on Electromagnetic Waves and Biology (Jouy-en-Josas, France), pp. 189-193, 1980.

Lebovitz, R.M. and R.L. Seaman. Microwave irradiation and instrumental behavior in rats: Unitized irradiation and behavioral evaluation facility. Bioelectromagnetics 1:415-428, 1980.

Lebovitz, R.M. Prolonged microwave irradiation of rats: Influence on concurrent operant behavior. Bioelectromagnetics. Bioelectromagnetics 2:169-185, 1981.

Lebovitz, R.M. and N.A. Nicholes. Computer based operant behavioral system for microwave bioeffects research. IEEE Trans. BME 28:536-543, 1981.

Lebovitz, R.M. Pulse modulated and continuous wave microwave radiation yield equivalent changes in operant behavior of rats. Physiology and Behavior (In press - 1982)



MICROCOPY RESOLUTION TEST CHART
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